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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

ANNUAL REPORT

U.S. NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

" Report of program activities.

(July 1, 1967 through June 30, 1968)

DIRECT OPERATIONS

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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
ANNUAL REPORT
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DIRECT OPERATIONS

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NICHD ANNUAL REPORT
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Office of the Director

The basic mission of the National Institute of Child Health and Human Development is to conduct and support research and research training in the processes of human development. The ultimate aim is to promote a nation of well-born individuals who will be able to achieve and maintain the optimum in physical, intellectual, and social health throughout continuingly productive lives.

During Fiscal Year 1968, the Associate Directors most deeply involved with the Institute's direct operations were extremely busy and productive.

The Associate Director for Program Planning and Evaluation and his staff are responsible for developing the data needed for the planning and the formulation of the Institute's major goals and objectives, and for the means for appraising progress towards these objectives. During the past fiscal year, the Associate Director increased his immediate staff to more adequately meet his responsibilities and organized his Office into five operational units: Planning, Evaluation, Planning-Budgeting, Interagency Liaison, and Legislative Liaison. With this increased staff, the Office prepared several documents describing the content, accomplishments, current activities, research opportunities, short-range objectives, and long-range goals of this Institute's programs. These included the Institute's contribution to the report on the National Institute's of Health and the Behavioral Sciences and to the NIH-wide report on Training Programs. Of considerable importance is the report on Optimal Health Care for Mothers and Children: A National Priority, a document which summarizes five meetings held with groups of consultants in the fields of obstetrics and gynecology, pediatrics, behavioral sciences, nursing, and social work. Other noteworthy activities of the Associate Director's office were the planning and development of a conference on the Acquisition and Development of Values and participation in the planning activities of the Program Planning Committees.

In addition, progress has been made with respect to the planning of intramural facilities. The shared NINDB-NICHD Reproductive Biology Research Facility at the Medical Center of the University of Puerto Rico is progressing satisfactorily with expected occupancy of the facility by July 1970. Planning for the Institute's Research Facility at NIH, a building of approximately 90,000 net square feet, is almost on schedule.

The past year was the first full year of operation of the Intramural Research Programs which were established by the Institute reorganization late in Fiscal Year 1967. Intramural Research Programs were given a firmly consolidated basis of operation on an independent footing during Fiscal Year 1968, a year marked by several significal developments. Of primary importance was the completion, occupancy, and dedication of the Gerontology Research Center Building at the Baltimore City Hospitals. Since the Intramural Research

Program must, in large part, structure its pattern of growth in accordance with the timetable on which space becomes available, the preponderance of Intramural Research Resources for FY 1968 were devoted to staffing and equipping the Center.

In other significant moves, the Children's Diagnostic and Study Branch, the Behavioral Biology Branch, and the Laboratory of Biomedical Sciences, which had previously cooperated in studies of mental retardation at the National Naval Medical Center, were given separate resources and independent bases of operation during the past year. Several important problems remain to be solved. Since the resignation of Dr. Loring Chapman to accept an academic post, the Behavioral Biology Branch has been without full-time leadership. The Children's Diagnostic and Study Branch, which has not had an adequate pool of subjects, is exploring the possibility of contract-supported activities at other institutions. In the past year, the Reproduction Research Branch made two attempts to establish a unit in a local teaching hospital with an obstetrical caseload which would be adequate to its clinical research needs. Since neither possibility materialized, the Branch plans a significant expansion of its current basic research activities in the endocrinology of reproduction as leased space becomes available.

The Laboratory of Biomedical Sciences has expanded significantly, clearly defined its mission, and established three new sections during the year. The Social and Behavioral Sciences Branch and the Developmental Biology Branch have not been notably expanded during the year. Space shortage continues to be a problem for both branches, and the planned growth of these units is dependent upon the timetable on which space becomes available.

During the year, the Intramural Research Programs of the Institute sponsored 26 scientific seminars at Bethesda and a weekly seminar, every Monday--September through May, at Baltimore. Regular monthly meetings with the Laboratory and Branch Chiefs were established. These, and other activities, have helped to define and promote the Intramural Research Programs of the Institute.

The Office of the Assistant to the Director for Population Research has continued to be responsible for Institute and interagency activities related to population research, excluding direct research conducted in the Institute's own laboratories and the research grants and other activities of the Reproduction and Population Research Branch. Of primary importance is the fact that the Institute has been designated the focal point at the National Institutes of Health for research in population control and family planning programs, and additional funds are being allocated for contract support in this area. It is planned to develop a contract instituting a recurring family growth survey whose primary focus will be knowledge, attitudes, and practices related to procreation and contraception. Another area of research receiving attention is the determinants and consequences of fertility patterns and the origins and effects of national population policies.

Additionally, a great deal must be learned about the psychological and social determinants and effects of contraceptive practices on parents, children, family relationships, sex behavior, and family stability. It has been argued

that the effective use of contraception may be one of the most powerful tools for promoting mental health, since it permits individuals to make effective and responsible decisions about an important part of their lives by removing sexuality from reproduction. These decisions warrant study. Abortion, though heavily laden with social and legal problems, is a legitimate subject for study. For instance, good data on the number of criminal abortions performed each year in the United States are not available.

The Epidemiology and Biometry Branch is primarily concerned with (1) providing consultative services in statistics and mathematics to both the intramural and extramural programs of the Institute and (2) carrying out research studies in statistical theory and methodology and in certain epidemiological areas related to the health mission of the Institute. During the past year, the Branch has been quite active in these areas. However, because of the lack of competent statisticians and epidemiologists available for recruitment, some activities and research programs planned in the previous year were not initiated. These include an in-house program of research in the epidemiology of mental retardation, the development of statistical methods appropriate for the analysis of data obtained in longitudinal studies, and the setting up of a more systematic operation for providing consultation and data analysis to the Gerontology Research Center.

The research activities of the Branch in epidemiology have been devoted primarily to investigations of the medical effects of oral contraceptives and other aspects of family planning. Several programs have been initiated and others are being planned, mostly through the contract mechanism. A member of the Branch is the coproject officer on contracts let to Kaiser Permanente, California; St. Mary's Hospital, London; and the UCLA, California. He is also the principal project officer on a contract let to the School of Public Health, University of California at Berkeley.

In addition to the research activities briefly mentioned above and its work with extramural areas of the Institute--mostly in a consulting capacity in the development of research studies, the Branch has provided advice and participation in the development of a clinical trial to decide whether high oxygen administration to newborns suffering from respiratory distress is more efficacious than low oxygen and monitoring the progress made in systematizing data for computer analysis under a contract issued to the Denver Child Research Council, Colorado.

Its activities in research in statistics during the past year include: (1) a new approach to the design of clinical trials; (2) detection of a change in a mean value over time; (3) contingency tables; (4) lifetable-versus-person-years analyses of data; and (5) the value of historical data in epidemiological studies.

The Office of Public Information concluded the year facing far-reaching changes in the Institute's approach to public information as a consequence of the delegation, to the National Institutes of Health, of public information functions previously carried out by the Public Health Service. As a result of this delegation, it became evident that all public information activities at the NIH would be reevaluated and reoriented during Fiscal Year 1969. This

reorientation will hopefully be an effective response to Congressional concern that the public become well informed concerning research advances.

Despite the fact that the Office of Public Information scored a number of successes in the public information field--notably with the major TV networks, an analysis of the workload of the Office indicated that approximately 55 percent of staff time was spent on internal reporting, 25 percent of staff time was spent on reporting to special (including scientific) publics, and only 20 percent of staff time was spent on public information activities.

A great bonus to the Institute has been information office cooperation with the major pediatric societies. OPI personnel assisted the American Academy of Pediatrics at both the annual and spring meetings. For the third year, the OPI planned and managed the press room for the pediatric research societies.

The Office of Program Services continued to explore ways in which the office's four branches could assist the Institute's direct operations. To this end, the Extramural and Contract Management Branch developed revised Institute policies and procedures for Institute contracts which were adopted by the Institute this year. Each of the three senior Grants Management Officers is assigned to an Institute program area and, in conjunction with the Program Director and/or his program staff, participates in the pre- and postnegotiation contract phases and in the execution of active contract management functions. Most of the work of the Program Statistics Branch is concerned with the extramural programs, but contracts and reimbursable agreements are now included in the Branch's machine data bank, and many of the reports and analyses prepared by staff of the Branch include contracts as well as grants. Intramural research is also included in some instances; for example, reports for the National Science Foundation, material prepared for use with Congressional committees, and special analyses requested by staff.

The work of the Scientific Conference Branch continued to make a significant contribution to the development of Institute programs. During Fiscal Year 1968, fifteen scientific conferences were held under the sponsorship and direct management of the Institute. Subjects of the conferences included principles of design for a perinatal research center; low-birthweight gaps in knowledge; sociological aspects of socialization; the reading process; nutrition and cell development; the interaction of retirement and health; socialization and retirement; health-related aspects of mental retardation; social science (family components) and mental retardation; and research technology (with the National Aeronautics and Space Administration).

The Scientific Information Centers Branch of the Office of Program Services now consists of five units: the NICHD Library and Translation Services; the Technical Publications Office; the Scientific Information Center for Adult Development and Aging; the Scientific Information Center for Growth and Development; and the Scientific Information Center for Reproduction and Population Research. The Scientific Information Centers' staff have been primarily involved in vocabulary development and in systems design and programming. The staff of the three Information Centers have completed and coded the biomedical, behavioral and social sciences, and related sciences

base vocabulary for the Adult Development and Aging and the Growth and Development Information Centers. The Reproduction and Population Research Information Center's special areas of the vocabulary are close to completion. In collaboration with the Information Retrieval Systems Unit of the NIH's Division of Computer Research and Technology, the staff has completed the development of the computer system for the publication aspects of the Information Centers' programs. An "International Directory of Gerontology" is being readied for publication with the target date of October 1968. During the past fiscal year, the principal activities of the Reproduction and Population Research Information Center were the publication of "Current Support of Research in Reproduction and Population" and "A Bibliography of Gonadotropin Literature, 1966." In the coming year, the staff, in collaboration with the Science Information Exchange, will prepare the next edition of the "Current Survey of Reproduction and Population Research." Work will also be started on publication of a journal covering the literature of reproduction and population.

The Technical Publications staff edited, prepared the bibliography for, and published "Patterns of Living and Housing of Middle-Aged and Older People" and edited and published "A Report on the Medical Exchange Mission to the USSR--Developmental Physiology and Pediatric Research." The Office is currently editing "The Reading Process," the proceedings of a conference sponsored by the Growth and Development Program; a tentative publication date of August 1, 1968, has been set. Two conferences on "low birthweight" are planned for the next fiscal year. The editor has designed covers for these books, compiled an extensive bibliography, and arranged for an index to be prepared.

Reference Library and Translation Service activities included filling 1,500 requests for books and periodicals from the Institute library, the NIH library, or by interlibrary loan. The Library has ordered Library of Congress cards and is setting up a subject catalogue. Four hundred and eighty-three pages of written translations from five (5) foreign languages were done by the translators as well as 50 pages of translations from letters.

A. INTRAMURAL RESEARCH

The Developmental Biology Branch. The long-term research program concerning menstrual and reproductive history, initiated by the Section Chief in 1934 at the University of Minnesota and continuously maintained by him since then, continues to be the major research activity of this Section. Because of lack of funds for certain new developments in data assembly, other aspects of the program are being emphasized. Intense exploitation of the great volume of data already on hand is proceeding along paths as planned. Statistical analyses of extensive data from unusually reliable sources which became available many years ago has led to the conclusion that errors in the date of LMP onset, as recorded routinely in hospital records, is not responsible for more than a relatively small part of the wide variation in the interval from LMP onset to birth. Reports on three different aspects of this problem have been completed, and a fourth paper, to conclude the series, is being developed.

The Reproduction Research Branch. The proposed and current research activities of the Branch, since its inception about 11 months ago, are outlined later in this report. In general, the program entails coordinated clinical and laboratory investigations of the reproductive process in man and animals. Whereas the initial emphasis is on the endocrinological phases of reproduction, it is expected that nutritional, genetic, and behavioral studies will be added as facilities may permit. Notwithstanding space limitations, the Branch has substantially extended the area of its clinical and animal studies. Especially noteworthy are the primate studies for which a very limited, but smoothly functioning, facility has been effectively utilized. On the whole, one can realistically expect that a well-rounded program of reproduction research will be evolved once the requisite clinical and laboratory resources can be specifically allocated. These will encompass (1) domicilliary care for women during all phases of pregnancy and (2) intrapartum and postpartum care appropriately coordinated with the prenatal and postnatal observations in the mother and newborn and with subsequent observations on pediatric growth and development.

Social and Behavioral Sciences Branch. The current research program of the Branch is focused on the infancy and preschool periods with a concentration on children from "disadvantaged environments." By studying extreme environmental conditions, it is hoped to delineate more sharply some of the variables significant to normal development. A major concentration of effort during the past year has been on the development and pretesting of methods for analyzing the components of preschool learning environments. Observations have been made in several types of preschool settings with different educational philosophies; e.g., Montessori, relationship-centered. During the past year, the Branch sponsored a conference on methodological issues in research on mother-infant interaction. Among the methodological issues considered were the relations between choice of observational categories and basic theoretical orientations, approaches to contingency analyses in observational studies, and basic problems of observer reliability.

Behavioral Biology Branch. This Branch is still in its very early stages of formation. Since the departure of Dr. Loring Chapman, who resigned as Chief of the Branch to accept the chairmanship of a university department, the four professionals currently working in the Branch have reported directly to the Scientific Director of the Institute. They have collaborated with the Children's Diagnostic and Study Branch in the electrophysiological and behavioral evaluation of children with disorders associated with mental retardation. In another collaboration, using pregnant sheep, they have gathered a considerable volume of data relating neurophysiological (EEG), cardiovascular, and metabolic observations on the fetus under hypoxia and during recovery from hypoxia. Expansion of the Branch and the development of its program awaits the appointment of a Branch Chief.

The Laboratory of Biomedical Sciences. This Laboratory investigates abnormal physical and mental development at several levels of organization. To further the objective of establishing a multidisciplinary environment which will foster this type of research, three sections have been formed during the past year: Section on Developmental Enzymology, Section on Intermediary Metabolism, and Section on Physiological Controls. The Laboratory needs further

strengthening in the areas of molecular structure, developmental genetics, and molecular biology. Among the more significant research results obtained in the Laboratory during the past year are: (1) cytogenetic examination of 172 abortuses revealed that triploid and XO aneuploidy are the most frequent chromosomal aberration encountered in spontaneous abortion specimens; (2) the regional and temporal distribution of four enzymes in the developing Rhesus monkey brain is being analyzed; and (3) the technique for the isolated perfusion of rat livers has been used to gain further insight into the mechanism of action of hormones at the molecular level.

The Children's Diagnostic and Study Branch. The Branch is an outpatient facility where research is conducted in the fields of medicine, biology, behavioral sciences, education, and epidemiology concerning various handicapping conditions such as mental retardation, learning disabilities, motor defects, and sensory deficits. Diagnostic studies are carried out on mentally retarded or physically incapacitated children using a multispecialty approach. However, the major bulk of the Branch's research projects involve subjects who are not included in the diagnostic studies. These include newborn infants at the National Naval Medical Center nursery and preschool- and schoolage children in Washington, D. C., and nearby counties as well as patients in institutions for the mentally retarded. In the past year, the clinic evaluated 94 children, including 31 who had been seen previously by the staff. As a result of the Branch's clinical and cytogenetic activities, a number of interesting patients have been encountered, such as a child with congenital sensory neuropathy or congenital anesthesia to pain, touch, and temperature who, in spite of his handicap, is intellectually and emotionally normal; and a four-and-one-half-year-old, mentally retarded child with monosomy G in the blood, skin, and bone marrow. To carry out some of the Branch's goals, inpatient facilities for satisfactory behavioral and biomedical observations and analysis are needed. The clinic is still faced with the problem of depending upon outside facilities in the performance of its routine activities such as neurological evaluation, EEG tracing, and biochemical assessment of biological specimens.

The Gerontology Research Center. Fiscal Year 1968 marked the completion of the Gerontology Research Building at the Baltimore City Hospitals. Occupancy of the new building was taken during the period February to May, and the Center was formally dedicated on June 15, 1968. During the year, an Instrumentation Section was established which has been of great value to investigators in all sections of the Gerontology Research Center. For example, a problem-solving computer has been designed and constructed which has greatly facilitated the administration of tests of problem-solving ability in the Section on Cognitive Behavior. The Center's prospective study of human aging continues to be characterized by the intensiveness and diversity of the studies performed on each visit, and by the good health and loyalty of the study group (only 5 percent have died, and almost 90 percent of those living remain active participants after approximately 10 years). Of critical importance for the analysis of aging trends in specific functions for individual participants is the fact that, of the 708 participants, almost 400 of them have been examined at least three times, 281 four times, and 166 five times. Accurate identification of individual aging patterns are difficult because of relatively high intrinsic measurement variance

associated with relatively low mean rates of change with age. Modern developments in biology have emphasized the importance of understanding the mechanisms of cellular processes in order to provide rational approaches to dealing with many of the diseases which play a role in impairments in the elderly, such as arthritis, vascular disease, diabetes, etc. Previous studies from the Gerontology Research Center have indicated the importance of the gradual loss of cells from many key tissues, such as muscles, nerves, kidneys, which takes place with aging and contributes to a gradual loss of reserve capacities in many organ systems. Hence, a part of the resources of the Center have been devoted to studies of cellular biochemistry and the structural characteristics of molecules involved in biological processes.

B. COLLABORATIVE RESEARCH AND CONTRACTS

During Fiscal Year 1968, the Institute processed 40 research contracts and reimbursable agreements for approximately $2.8 million. Of these, 20 of the contracts and reimbursable agreements--valued at $1.4 million--were continuing and 20--valued at $1.4 million--were new. This program represents a net increase of 4 contracts and reimbursable agreements and $0.2 million over the Fiscal Year 1967 program.

Population Research. The first area of emphasis in population research--reproductive biology--was represented with only one contract. This was for a symposium held at Washington State University on the mammalian oviduct. In the second area of emphasis in population research--contraceptive methodology, the Institute negotiated a large contract with the Kaiser Foundation Research Institute. The purpose of the project is to undertake a long-term study of a variety of medical effects of oral contraceptives in women. The study population comprises 20,000 women, half of whom are expected to be on oral contraceptives and half using other methods of contraception. While some data generated by the study will be available shortly, information on the long-term effects of oral contraceptives, such as the possible relationship between their use and carcinoma of the breast or cervix, will require several years. Other contracts to study oral contraceptives are somewhat more restricted in nature. The lease well-developed area of population research is family planning and population planning. This involves individual, interactional, familial, institutional, and societal processes. Currently, only two contracts in this area are being funded: one is the continuation of a survey of American family planning practices conducted by Princeton University, and the other is for the continued support of the training and experience of physicians in the epidemiology of fertility regulation through the National Communicable Disease Center in Atlanta.

Perinatal Biology and Infant Mortality Branch. The Perinatal and Infant Mortality Branch is supporting four contracts. The first, with the Children's Hospital Research Foundation, Washington, D. C., is for a study of unexpected infant death in the Washington, D. C., area, with particular emphasis on upper respiratory viral infection as a possible cause of sudden infant death. The second, with the University of Oregon Medical School, is to develop a Pygmy goat colony as a resource for research purposes. The third, with Columbia University, is for a document concerned with the diagnosis and

treatment of disorders affecting the intrauterine patient. The fourth, with Group Health Association, Inc., is preparing a scientific report on specific aspects of the psychodynamics of pregnancy. A major gap in the area of perinatal and infant survival and well-being concerns psychosocial factors of pregnancy and their relationships to pregnancy outcome and perinatal and infant mortality. It is anticipated that this report, based on the analysis of data from the Association's Demonstration Project on Prenatal and Early Postnatal Adaptation, will reveal new knowledge on the psychological adaptation to pregnancy and its relationship to pregnancy outcome and subsequent maternal-infant adaptation.

Growth and Development. During the past 12-month period, special attention was given to utilizing research contracts for meeting specific targeted goals of the Growth and Development Program. Thus, the total number of contracts administered by Branch staff has increased from 9 in FY 1967 to 15 at the present time. A variety of projects, ranging from the development of nutrition terminology for literature storage and retrieval purposes to improved methodologies for studying how children and youths learn to live in the world about them have been implemented. At the same time, improved monitoring of existing projects has been a major goal of the staff. The Institute's largest single contract, devoted to the study of malnutrition and mental development, has just been reviewed in depth by international experts and endorsed as a major interdisciplinary study in an extremely critical area. One conference was supported by contract during the last year. This weeklong symposium on "Issues in Human Development" brought together biological and behavioral scientists with specialists in urban planning and law to discuss many of the leading issues facing the United States today. The proceedings are being edited for publication next year.

Because existing longitudinal data should be fully analyzed and interpreted before initiating new studies, a significant contract has been implemented with the University of Colorado to provide support for the organization and analysis of anthropometric, radiographic nutritional and health history data obtained on some 200 subjects over the past 36 years by the Denver Child Research Council. It is anticipated that the results of this contract will serve as a model for other projects involving large-scale longitudinal child development data analysis.

Mental Retardation Branch. The four contracts which the Mental Retardation Branch funded during 1968 were concerned with the following: (1) regional cooperation in mental retardation research, prevention of mental retardation: the Lesch-Nyhan Syndrome, (3) a study of medical care practices in institutions for the mentally retarded, and (4) an experimental study of mental retardation classification system. Data collected from the contract on regional cooperation now include 20 state institutions and more than 26,000 patients. The population profiles now available offer rich resources for epidemiological and other type studies. The second contract, on the Lesch-Nyhan Syndrome, supported a conference cosponsored by the Federation of American Societies for Experimental Biology in October 1967. The third contract, on medical care practices, is for a developmental study to lay the foundation for a broad-scale assessment of medical care practices in institutions for the retarded. The significance of the project lies in

its usefulness in providing an informed basis of the factors influencing medical care for national and local planning and for the development of standards. The final contract, concerned with classification, is with the American Association on Mental Deficiency. The initial phase of the contract has helped to clarify existing problems in the reliability and validity of the current classification system and to identify difficulties encountered in the use of the system.

Adult Development and Aging Branch. The Adult Development and Aging Branch supported only two contracts this past year. One was for the publication of "Current Publications in Gerontology and Geriatrics," with the Gerontological Society. The objective of this contract is to assemble a bibliography of research in the field of aging consisting of approximately 3,000 citations and to publish this quarterly, "Current Publications in Gerontology and Geriatrics," in the Gerontological Society's publication, "Journal of Gerontology." The second contract, with the American Institutes for Research, is concerned with the theoretical aspects of retirement. This activity is aimed at the development of several theoretical models descriptive of the retirement process. The models will consider several aspects of retirement including the biological, psychological, and social. It is anticipated that they will serve to guide research on retirement by providing theories which research can then test. The retirement process and its related physiological, psychological, and social factors is an important area of study and may well be basic in the development of many programs in social gerontology.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Office of the Assistant to the Director for Population Research

I. Definition of the field and establishment of goals.

Population research is defined broadly since disproportionate population growth creates problems in many phases of life and requires the application of a variety of disciplines to find solutions. The Institute's approach is discussed under the following topics: reproduction, contraceptive methodology, and family and population planning.

A. Reproduction.

Population research in reproduction has two aspects: reproductive biology and reproductive behavior.

1. Reproductive biology. The processes of reproduction are remarkably intricate and require the application of all fields of biology. Emphasis is placed on a better understanding of the reproductive process which will allow for the development of an array of effective contraceptives.

2. Reproductive behavior. This includes the psycho-physiological factors involved in reproduction and its control and the social and psychological determinants and consequences of reproductive and non-reproductive sexual behavior, and the decision-making processes involved. These include behavioral studies of reproduction and mating as well as the psychological effects of crowding and population density; effects of attitudes, values, norms, motivation, incentives and sanctions on human reproductive behavior in various groups; and the psychological and social influences on marriage, childbearing and spacing, desired and actual family size and infertility.

B. Contraceptive methodology.

No presently available method of contraception fulfills the criteria of effectiveness, safety, low cost, and acceptability to various population groups. The oral steroids and intrauterine devices represent remarkable improvements in contraceptive technology, but it is well recognized that they do not fulfill all these criteria. Probably no single method will be universally satisfactory in all situations. The development of an array of methods of contraception and the evaluation of the safety and efficacy of methods in use is the goal of this area of population research.

C. Family and Population Planning.

Studies of the issues of population control are supported. These include attitudes, values, norms, motivations, incentives, sanctions, and decisions that affect contraceptive practice and choice of method of various socio-cultural, ethnic and economic groups at the individual and societal levels.

Other aspects of the program include studies of the effects of contraceptive practices, child spacing and birth limitations on children and on the roles of men and women; conditions for and the consequences of the development of effective population programs; and cross-cultural studies of motivational factors, propaganda methods, and communications for the distribution of family planning programs.

Research in population dynamics including computer simulation of past, present and projected population growth rates and of population composition and distribution will be supported along with studies of effects on social structures and economic growth both in developing and technologically advanced countries. Improved demographic techniques will aid in the design and evaluation of family planning programs and the determination of optimal target groups.

II. NICHD Population Research Program: Collaborative Research and Contracts.

See section on Contracts and Collaborative Research

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968
Office of Associate Director for Program Planning and Evaluation
(Summary)

The Associate Director for Program Planning and Evaluation and his staff are responsible for developing the data needed for the planning and the formulation of the Institute's major goals and objectives, and the means for appraising progress toward these objectives. In performing these functions, this Office:

(1) Sponsors and conducts studies on the nature of the emerging problems and needs within the broad field of child health and human development; (2) stimulates and provides guidance for the analysis of the status and character of scientific activity in problem areas, the extent of institute program involvement therein--the resources available and needed for further program efforts; (3) coordinates activities leading to the selection of program objectives and the development of program plans reflecting the decision of the Institute directorate; (4) prepares and maintaines a comprehensive multi-year program plan utilizing the planning-programming-budgeting system and other analytical techniques; (5) sponsors and conducts reviews of program activities to assess progress toward objectives and to evaluate accomplishments as basis for the further development or modification of institute plans; and (6) maintains an awareness of ongoing Congressional activities, keeps staff informed of legislative developments, and coordinates matters of mutual concern with other interested governmental and non-governmental organizations.

During the past fiscal year, Dr. Dwain N. Walcher, Associate Director for Planning and Evaluation, has increased his immediate staff to more adequately perform the functions stated above. The Office has been organized into five operational units as follows: Planning, Evaluation, Planning-Budgeting, Inter Agency Liaison, and Legislative Liaison. The immediate staff currently consists of a physician responsible for clinical planning; a veterinarian responsible for biological evaluation; a program analysis officer to keep informed of legislative activities; a program analyst to assist in behavioral science evaluation; and a program analyst to assist in implementing the planning-programming-budgeting system. The program planning officer has been attending classes at Stanford University for the past year and will be returning to the office in August. By July 1968, the Associate Director will add to his staff a physician responsible for clinical evaluation; a physician responsible for planning manpower and training needs; and a sociologist for behavioral science evaluation.

In fiscal year 1968, this office prepared several documents describing the content, accomplishments, current activities, research opportunities, short-range objectives and long-range goals of this Institute's programs.

A report on the development of NICHD training programs was submitted for inclusion in the report entitled: The Development of NIH Training Programs to Meet National Needs for Research and Training in the Biomedical Science.

A report on NICHD's role in the behavioral sciences was submitted for inclusion in a document entitled: NIH and The Behavioral Sciences which was prepared in response to a report submitted by the sub-panel of the Advisory Committee to the Director, NIH.

A report was prepared in response to a request from the Office of Assistant Secretary for Health and Scientific Affairs, DHEW, which suggested specific areas for population research in P.L. 480 countries utilizing the disciplines within the behavioral and/or biological sciences.

Optimal Health Care for Mothers and Children: A National Priority is the title of a document which sumarizes five meetings held with groups of consultants in the fields of obstetrics and gynecology, pediatrics, behavioral sciences, nursing and social work, at the request of the Secretary and the Surgeon General, in an attempt to learn how manpower and other needs in the child health field can best be met.

A five-year program plan of goals and objectives for the Institute's Center for Population Research was developed in accordance with the planning-programming-budgeting system in order to achieve effective and efficient management of this new Center which was proposed in the President's Health Message.

A report describing special, organized programs within the Institute warranting particular consideration was submitted for inclusion within the NIH preliminary Program and Financial Plan, 1970-74.

A conference on the Acquisition and Development of Values was held in May for the purpose of advancing the frontiers of social science research by defining the determinants of moral values, ethical standards, and character formation by delineating those institutions of living which influence personality and value development and by defining those scientific disciplines which can contribute to and improve studies of these factors.

This Office has actively participated in the planning activities of the Program Planning Committees. Staff members functioned as executive secretaries for the Program Planning Committees which met in November and prepared summary reports in four program areas: Perinatal Biology and Infant Mortality; Growth and Development; Adult Development and Aging; and Mental Retardation. Currently, the Program Planning Committees are directing their efforts to the development of position papers in three major areas of concern. The Population Research Program Planning Committee is preparing a paper dealing with fertility control; the Perinatal Biology and Infant Mortality Program Planning Committee is preparing a paper on respiratory distress syndrome, and the three program areas - Growth and Development, Adult Development and Aging, and Mental Retardation have joined forces to prepare a paper on psycho-social deprivation.

Liaison is maintained with the Division of Research Facilities and Resources with regard to planning involving grant support for the construction of new health research facilities.

Progress has been made with respect to the planning of intramural facilities. Our Gerontology Building in Baltimore was completed in the spring. The shared NINDB-NICHD Reproductive Biology Research Facility at the Medical Center of the University of Puerto Rico, which will also include a monkey breeding and social enclosure facility ten miles away at Sabana Seca, is progressing satisfactorily with expected occupancy of the Sabana Seca facility by the fall of this year. Planning for the NICHD Research Facility at NIH, a building of approximately 90,000 net square feet, is almost on schedule and as of this writing should permit construction to begin in early spring of 1969 and occupancy in the early fall of 1971. Tentative drawings should be ready for formal review in June.

ANNUAL REPORT
Office of Public Information
July 1, 1967 through June 30, 1968

Fiscal year 1968 may be remembered as the year that the major television networks discovered NICHD. This discovery was prompted by media interest in the early years of life, infant mortality, and problems of the fetus--all areas of intense NICHD activity.

The year began and ended with major efforts by CBS-TV. The first was "Mystery of Life" on Walter Cronkite's "21st Century" series, featuring several NICHD grantees working in reproduction and embryology. Later that year, the OPI helped CBS with a program on early education by arranging interviews with several staff members and naming grantees working in this field. In May, a CBS production assistant spent several days at the Institute and at the Institute press room at the Pediatric meetings in Atlantic City, interviewing experts in the fields of intra-uterine life and care of the newborn. This effort was for a program to cover the first ten months of life and will point out that a new type of patient--the fetus--will receive the attention of a new type of medical specialist--the fetologist.

"How Life Begins," the first comprehensive telecast on the reproductive processes, was shown on ABC network television. The OPI worked closely with the producer of this program during the early planning stages. Scientists holding NICHD research awards were interviewed and results of their studies were included in the telecast.

Work being done at the Institute's Gerontology Research Center in Baltimore was included in the program, "Can We Live To Be 100?," broadcast nationally on the CBS "21st Century" series. Center research dealing with the effects of nutrition and environment on life span was highlighted, along with several of the physiological and psychological longitudinal studies involving volunteers.

A two part program on early learning was presented by CBS in February. "The 21st Century" staff interviewed several behavioral scientists at NICHD in preparation for the program which featured several NICHD grantees.

In addition to these already televised programs, NBC representatives have been discussing plans for an hour-long documentary on early life.

The effect of such efforts in assisting the television networks is difficult to assess. Most often the investigator being shown is given credit but his source of support is not. However, the public interest created by these programs is certainly of value to the Institute in creating general awareness of Institute programs and career potential in areas of Institute responsibility.

A great bonus to the Institute has been information office cooperation with the major pediatric societies. A staff member assisted the American

Academy of Pediatrics in running the press room and providing advance TV coverage of the annual and spring meetings. As a result, the Academy has furnished the Institute with mailing lists and has offered consultation on press projects. In addition, the press room activity permitted contact with reporters covering the child care aspects of pediatrics. The OPI, for the third year, planned and managed the press room for the American Pediatric Society and the Society for Pediatric Research at their annual joint meeting in Atlantic City. This meeting invariably generated a lot oa press interest, both at the meeting and in subsequent follow-up publicity about NICHD programs.

Interest in aging research remained high during the year. Early in the year, the OPI produced a press release concerning housing for the aged, with subsequent newspaper and magazine interest. Lengthy articles appeared in Business Week, JAMA Medical News, and Drug Research Reports. The major activity in gerontology has centered around the opening of the new Gerontology Research Center building in Baltimore. The establishment of a permanent satellite information office at the Center facilitated planning and carrying out the opening ceremonies. Press previews and tours as well as a working press room were procided for the dedication ceremonies.

In addition to the pediatric and gerontology activities, the OPI planned and staffed a press room in the spring in connection with the NICHD-sponsored conference on "Studies in the Acquisition and Development of Values," held at the Pan American Health Organization building in Washington.

The Institute's activities in the field of population and reproduction continues to be of year-round interest to the communications media. The year started out with three OPI press releases on contracts to study the medical aspects of oral contraceptives, setting the tone for the rest of the year both in press and public interest. Later an FDA report on IUDs and announcement of proposed restudy of oral contraceptives sparked interest again in Institute programs. Continuing concern over the problems of oral contraceptives in the mass media kept reporters calling for background material and news.

The OPI performs many services for Institute programs, some requiring professional judgment and skills and others organizing clerical tasks.

In preparation for appropriation hearings, the OPI prepared the Director's opening statement, a 20-page progress report, highlights of research, and a special report on aging research. The OPI also administers the formal clearance procedures for manuscripts and speeches produced by Institute staff. This task involves checking to see that papers are prepared in conformance with NIH regulations, preparing clearance forms, and expediting movement of papers through the clearance process. In FY 1968 more than 180 speeches, abstracts, and papers will have been cleared.

The OPI answers press, public, and Congressional inquiries concerned with Institute problems. These inquiries come directly to the Institute or are referred from other parts of the Federal government. While inquiries that require individual answers have remained constant in the past two years, about 3000 per year, the number of inquiries that can be answered by booklet

or form letter have greatly increased. As a result, the OPI has drafted form letters and selected bibliographies for students doing papers on abortions, birth control, and birth defects, topics that seem to be very popular with high school and college teachers.

Other Projects of Interest

The OPI assisted West German TV in making a documentary film on the Children's Diagnostic and Study Branch. The OPI later assisted in producing English sound track and new titles for this film, which is now used as an information film by this Institute Branch.

The OPI assisted the Institute's Director in preparation of a lengthy question and answer interview article which appeared in two successive issues of OB/GYN News.

Five new program pamphlets refelcting changes in NICHD organization and program emphasis were completed and distributed. These brief pamphlets, containing pictures and text, are the main source of general information about the types of research supported by the Institute.

The OPI edited and helped produce two books for the Institute, Patterns of Living and Housing of Middle Aged and Older People and The Prevention of Mental Retardation Through Control of Infectious Diseases. Publicity and distribution of these books was also handled by the OPI.

The Growth and Development Exhibit was shown at the American Psychological Association meeting in Washington. The Institute's new exhibit on infant mortality had successful showings at the American Academy of Pediatrics, AAS, the Annual Meeting of the American College of Obstetricians and Bynecologists, and the Annual Meeting of the American Medical Association. This is the first Institute exhibit that is suitable for showing at both clinical and scientific meetings.

New treatment of the newborn was the topic of several stories for which the OPI furnished interviews and material. Look magazine did a story on infant mortality; New York Times Magazine did a round-up story on fetal surgery and prenatal life; McCall's story on "Protecting the Unborn Baby" featured NICHD grantees working on Rh sensitization.

Plans

Audiovisual activities need to be strenghtened even more. Careful planning and thoughtful presentations to the major TV networks encourages national exposure to problems of concern to the Institute. The potential for audiovisual presentation of Institute programs has not yet been thoroughly explored due to lack of personnel capable of carrying out such a program.

Attaching other information personnel to specific programs as at the Gerontology Research Center will be needed as programs get larger and expand. Information personnel are particularly essential in high priority programs in order to keep track of day-to-day developments and respond to requests.

The OPI needs to be able to take advantage of contract writers for long-term writing projects. The staff is of such a size now that tying up staff members in long-term projects severely reduces the OPI's effectiveness in dealing with the more urgent problems of assisting the press, covering developing stories, and answering emergency requests.

NICHD Annual Report
July 1, 1967 through June 30, 1968
Epidemiology and Biometry Branch

The Epidemiology and Biometry Branch is primarily concerned with 1.) providing consultative services in statistics and mathematics to both the intramural and extramural programs of the Institute, and 2.) carrying out research studies in Statistical Theory and Methodology and in certain epidemiological areas related to the health mission of the Institute. During the past year, the Branch has been quite active in these areas. However, because of the lack of competent statisticians and epidemiologists available for recruitment, some activities and research programs planned in the previous year were not initiated. These include an in-house program of research in the epidemiology of mental retardation, the development of statistical methods appropriate for the analysis of data obtained in longitudinal studies and setting up a more systematic operation for providing consultation and data analysis to the Gerontology Research Center. The problems encountered in recruiting staff are frustrating and discouraging. It probably constitutes the most serious limiting factor in instituting programs necessary to fulfill the mission of this Branch.

The research activities of the Branch in epidemiology have been devoted primarily to investigations of the safety of Oral Contraceptives and other aspects of Family Planning. Several programs have been initiated and others are being planned, mostly through the contract mechanism. A member of the Branch is co-project officer on contracts let to Kaiser Permanente, California; St. Mary's Hospital, London; and to the UCLA, California. He is also the principal project officer on a contract to the School of Public Health, University of California at Berkeley. He has also participated in the planning of additional studies (listed under Consulting Projects).

Research in Epidemiology

As indicated in last year's Annual Report, the interest of the Branch in developing these studies with outside investigators, in addition to furthering the research objectives of the Institute, is to make available to the Branch data collected in these studies for careful analysis. During the year, the Branch has been asked to analyze data collected by the Worcester Foundation on side effects incurred with the use of oral contraceptives. Punch cards have been received and the information put on tape. Editing of the data is now in process. At the present time, computer programs are being developed to complete the edit and to analyze the data. Of particular interest will be the incidence of side effects, correlations among side effects and their clustering patterns within subjects. It is also planned to perform life table and person year analyses.

In the area of Mental Retardation, the Branch Chief met with the Head of the Epidemiology Department at Columbia University in order to pursue some research studies of mutual interest. He worked closely with a member of the Mental Retardation Program in the extramural area in having a proposal

developed. A contract proposal has been submitted to the Institute. The research proposed is an experimental epidemiological study in a section of Harlem, New York City, to determine whether a protein supplement added to the diet of pregnant women can decrease the incidence of low birthweight babies (the frequency in Harlem is about 17%).

In addition, the Branch has been working closely with other extramural areas of the Institute mostly in a consulting capacity in the development of research studies. In particular, the Branch has provided advice and participation in:

1. The development of a clinical trial to decide whether high oxygen administration to new borns suffering from respiratory distress is more efficacious than low oxygen, and,

2. Monitoring the progress made in systematizing data for computer analysis under a contract issued to the Denver Child Research Council which has collected longitudinal data in growth and development over the past thirty years.

Research in Statistical Theory and Methodology

During the past year the members of the Branch have been actively engaged in research in Statistics proper. Some of these activities are listed below:

1. A new approach to the design of clinical trials. A new procedure for the planning of clinical trials has been investigated in collaboration with two other statisticians in the NHI. This research has been primarily in statistical theory using a Bayesian approach to inference and permits the modification, during the course of the trial, of the number of individuals to be assigned to the inferior treatment as information accumulates that one treatment is indeed inferior. The impact of such a procedure on ethical problems surrounding clinical experimentation is obvious. A manuscript has been submitted for publication.

2. Detection of a change in a mean value over time. This is important in the analysis of longitudinal studies. This research was begun during the year and is now continuing.

3. Contingency Tables. The analysis of r x c classification tables are customarily done by Chi-square. Difficulties arise, however, when several of the expected cell frequencies are small or zero. The Branch is investigating the use of an Information Theoretic Statistic.

4. Life Table versus Person Years Analyses of Data. In order to investigate the hypothesis that the person years technique yields an overestimate of risk per individual, the Worcester data previously mentioned will be used to compute and compare estimates of risk obtained by this approach and by the life table technique.

5. The value of historical data in epidemiological studies. The conventional techniques used in epidemiological studies to determine the effect of some agent on a disease is either a prospective or retrospective approach. However, information on the risk factor is clearly present in the trend of the incidence of the disease in the community in the years following the introduction of the agent. The questions to be studied are: 1.) How should one estimate the relative risk? and, 2.) How powerful a procedure is the historical data statistically?

Consultative Services

As indicated earlier, a major activity of the Branch is providing consultation to scientists within the Institute and outside of NIH. As the intramural research activities of the Institute expand, it is planned to establish a Section of Experimental Statistics with the sole purpose of advising intramural experimenters on design and analyses of experiments and on any aspect of biomathematics including the development of mathematical models to describe biological mechanisms. These models may be either of a deterministic or stochastic nature. During the year, advice on design and analyses problems has been provided to several scientists in the Intramural Program.

During the past year, the three members of the Branch have been consulting with many scientists outside of the NIH who have been contacted by Institute program staff to develop research proposals in the various areas of interest. In many instances these have involved working closely with the investigator discussing aspects of design, sampling, methodology such as interview techniques, collecting and recording of data, matching versus non-matching, effective controls, follow-up techniques, etc., and methods of data analysis including the use of computers.

A selection of some of the major consulting projects follows:

1. Consultations on design and planning problems with members of the Children's Diagnostic and Study Branch. The research questions concerned the detection and study of defects in outcome of pregnancy in certain selected high risk pregnancies.

2. Design of studies on Downs syndrome and other congenital abnormalities with members of the Children's Diagnostic and Study Branch.

3. A proposal from Downstate Medical Center in New York City to assess frequency of congenital malformations and other defects occurring in births to women using oral contraceptives. This study was developed from a previous proposal from Planned Parenthood Association.

4. A case-control study being discussed with the State Health Department of Connecticut on breast cancer in women using oral contraceptives.

5. Kaiser Permanente, Walnut Creek, California. The relationship of oral contraceptive use and subsequent health of women studied in a population whose medical care is well recorded.

6. A study conducted by the Epidemiology Department of the School of Public Health at Berkeley, California, to determine factors which cause failures in the use of intra-uterine contraceptive devices.

7. A proposal from St. Mary's Hospital of the University of London to study metabolic and other physiological changes in women using oral contraceptives.

8. A project from the School of Public Health, UCLA, to study the patterns of progressive changes of dysplasia of the cervix occurring in women using oral contraceptives.

9. Analysis of data collected in a study of the modification of human pain tolerance and the design of an experiment relating to questions on the attention of infants with a member of the Behavioral Biology Branch.

10. A cooperative pediatric clinical trial with the core group at the Cleveland Metropolitan General Hospital. This planned trial is to determine whether a certain treatment is significantly more efficacious than the standard procedure in treating respiratory distress syndrome (Hyaline Membrane Disease) in new born infants.

11. The logistical problem of setting up a system of data flow on to the computer and the problems of statistical analysis in the Denver Child Research Council longitudinal study on growth and development.

12. Continuing consultation on analysis of data with various staff members of the Gerontology Research Center in Baltimore.

Activities of Members of the Branch

The Branch Chief

1. Presented an invited lecture to the Statistics Department, Princeton University, February 1968.

2. Presented three National Science Foundation lectures in statistics:

   a) Mathematics Department, PMC College, Chester, Pa., February 1968.

b) Mathematics Department, York Jr. College, York, Pa., February 1968.

c) Applied Mathematics Department and Computer Sciences Division, University of Virginia, Charlottesville, Va., March 1968.

3. Presented an invited paper at the Spring Joint Meetings of the Biometric Society and The Institute of Mathematical Statistics: "An Adaptive Procedure for Sequential Clinical Trials", VPI, Blacksburg, Va., April 1968.

4. Presented an invited lecture to the Statistics Department, State University of New York at Buffalo, May 1968.

During the past year, the Branch Chief continued to serve on the NIH Ad Hoc Study Section in Biostatistics and Biomathematics, on the NIH Biostatistics Fellowship Review Committee, on the Committee reviewing the validity of the data base of Perinatal Collaborative Research Project (NINDB), and as Associate Editor of the Journal of the American Statistical Association.

During the year, he was appointed to the HEW Career Service Board for Mathematicians and Statisticians, to the FDA Epidemiology and Biometry Advisory Committee, and to the NCI Biometry and Epidemiology Contract Review Committee.

The Branch Chief has been elected President-Elect of the Biometric Society, Eastern North American Region, 1968, to serve as President in 1969. He also was President of the Washington Statistical Society in 1967-68.

Dr. Seigel

1. Presented an invited paper at the annual meeting of the American Public Health Association in Miami, Florida, October 1967.

2. Presented an invited paper on "Structured Retrospective Studies" at a Symposium on Adverse Drug Reactions at Atlantic City, May 1968.

Dr. Seigel continued to serve on the Editorial Board of Harvard University School of Public Health Alumni Bulletin.

Branch Publications

1. Cornfield, J., and Greenhouse, S.W.: On certain aspects of sequential trials. In LeCam, L., and Neyman, J. (Eds.): Fifth Berkeley Symposium on Mathematical Statistics and Probability, 5: University of California Press, Berkeley, Calif., 1967, pp. 813-829.

2. Bartko, J.J., Greenhouse, S.W., and Patlak, C.S.: On expectations of some functions of Poisson variates. Biometrics. 24: 97-101, Mar. 1968.

3. Greenhouse, S.W.: The meaning of discrimination classification mixture, and clustering in statistics. In Katz, M.M., Cole, J.O., and Barton, W.E. (Eds.): The Role and Methodology of Classification in Psychiatry and Psychopathology. Washington, D.C., 1968, pp. 197-201.

4. Mantel, N., and Greenhouse, S.W.: What is the correction for continuity? The American Statistician. In press.

5. Markush, R.E., and Seigel, D.: Prevalence at Death I. A new method for deriving death rates for specific diseases. A.J.P.H. 58: No. 3, 544-557, Mar. 1968.

6. Seigel, D., and Markush, R.E.: Prevalence at Death II. Methodological considerations for use in mortality studies. A.J.P.H. 58: No. 4, 772-776, Apr. 1968.

7. Seigel, D., and Corfman, P.: Epidemiological problems associated with studies of the safety of oral contraceptives. JAMA. 203: No. 11, 950-954, Mar. 1968.

8. Seigel, D., and Markush, R.E.: The interpretation of incomplete responses to a mailed epidemiological inquiry. Arch. Environ. Health. 16: No. 3, 420-423, Mar. 1968.

9. Seigel, D., and Krueger, D.E.: Consistence of incidence, survival, and prevalence data on myocardial infarction from various studies. J. Chron. Dis. 20: 603-614, Aug. 1967.

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Office of Associate Director for Program Services
Direct Operations

The Office of Program Services continues to explore ways in which the office's four Branches can assist the Institute's direct operations. The major contribution this year has been in the Institute's contract program as described in the following two Branch reports. The Extramural and Contract Management Branch developed revised NICHD policies and procedures for NICHD contracts which were adopted by the Institute this year.

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Grants and Contracts Management Branch

Summary

Although the bulk of this Branch's activities involve the grant and award programs of the Institute, it also has responsibility for the administrative management of the Institute's contract program; i.e., the non-scientific aspects of the contracting process. Each of the three senior grants management officers is assigned to an Institute program area and in conjunction with the Program Director and/or his program staff participates in the pre- and post-negotiation contract phases and in the execution of active contract management functions.

During FY 1968, we expect to process 40 research contracts and reimbursable agreements for approximately $2.8 million. Of these, 20 of the contracts and reimbursable agreements valued at $1.4 million are continuing and 20 valued at $1.4 million are new. The FY 1968 program represents a net increase of 4 contracts and reimbursable agreements and $0.2 million over the FY 1967 program.

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Program Statistics Branch

Most of the work of the Program Statistics Branch is concerned with the extramural programs, but contracts and reimbursable agreements are now included in the Branch's machine data bank, and many of the reports and analyses prepared by staff of the Branch include contracts as well as grants. Intramural research is also included in some instances; for example, reports for the National Science Foundation, material prepared for use with Congressional committees, and special analyses requested by staff.

NICHD had 38 active research contracts (including reimbursable agreements) as of April 30, 1968. Total funds obligated for these active contracts came to 2.9 million dollars, the great bulk of which represented the Reproduction and Population Research and Growth and Development programs.

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Scientific Conference Branch

Fifteen scientific conferences were held during FY 1968 under the sponsorship and direct management of this Institute. Such conferences are used as a medium to bring together active scientists from a variety of bio-medical and social science backgrounds to discuss progress in research relative to the objectives of NICHD.

The conferences by program area and subject were as follows:

Perinatal Biology and Infant Mortality

Principles of Design for a Perinatal Research Center
December 1967 at Yale

Low Birth Weight - Gaps in Knowledge
2 Conferences - June 1968 - Hollywood, Florida

Growth and Development

Sociological Aspects of Socialization
December 1967 - Washington, D.C.

The Reading Process
February 1968 - New Orleans, Louisiana

Nutrition and Cell Development
May 1968 - Asheville, North Carolina

Adult Development and Aging

The Interaction of Retirement and Health
December 1967 - New Orleans, Louisiana

Socialization and Retirement
February 1968 - San Francisco, California

Mental Retardation

Health Related Aspects of Mental Retardation
October 1967 - Washington, D.C.

Social Science (Family Components) and Mental Retardation
January 1968 - Bethesda, Maryland

Research Technology (with NASA)
February 1968 - Houston, Texas

The fourth and fifth conferences in the series on Optimal Health Care were held by the Office of Program Planning and Evaluation in August and December 1967, respectively. A summary report of the five conferences, entitled Optimal Health Care for Mothers and Children: A National Priority, was developed, carefully reviewed by the participants and made available for wide distribution to pediatricians, obstetricians, social workers, nurses, psychologists and others concerned with research and training programs for the health of children and their families.

The conference on the Acquisition and Development of Values held in May 1968 at the Pan American Health Organization Building in Washington, D.C. was the culmination of two years of intensive planning. The conference combined the advantages of a scientific forum and a working group by interrelating formal presentations with discussions among panelists from a wide spectrum of social science disciplines. The participants and hundred plus observers provided broad representation of professional, geographic, ethnic and age group differences. A report of the conference is in preparation.

The Branch also provided logistic support to the meetings of the Program Planning Committees and several other small Institute supported groups.

Consultation on conference planning was given to individuals from other agencies of Government and non-governmental organizations in response to requests.

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Scientific Information Centers Branch

The Scientific Information Centers Branch now consists of five units:

1. The NICHD Library and Translation Services
2. The Technical Publications Office
3. The Scientific Information Center for Adult Development and Aging
4. The Scientific Information Center for Growth and Development
5. The Scientific Information Center for Reproduction and Population Research

SCIENTIFIC INFORMATION CENTERS STAFF ACTIVITIES:

Vocabulary Development:

The staff of the three Information Centers has completed and coded the Biomedical, Behavioral and Social Sciences, and Related Sciences Base Vocabulary for the Adult Development and Aging and the Growth and Development Information Centers. The Reproduction and Population Research Information Center's special areas of the vocabulary are close to completion. The Control Parameters vocabulary has been completed and coded for use by all the Centers.

Systems Design & Programming:

In collaboration with the Information Retrieval Systems Unit of the NIH Division of Computer Research and Technology, the SINFCB staff has completed the development of the computer system for the publication aspects of the Information Centers programs. The language used in programming is FORTRAN IV for the IBM 360 Model 50. The staff is also investigating a new input model, the magnetic tape devices and a new editing device, the cathode ray screen coupled with a light pen device. The retrieval programs are in the design stage.

Cooperation with Other Organizations:

The staff met with representatives of the Education Research Information Centers (Office of Education), NINDB, NIAMD and the National Library of Medicine to arrange methods of information exchange and vocabulary compatibility.

Attendance at Meetings:

The Chief and Assistant Chief of the Branch discussed the Adult Development and Aging Information Center and its services with the Executive Committee of the President's Council on Aging on May 7, 1968.

The Chief and Assistant Chief of the Branch were invited to a seminar on

April 26, 1968 at Catholic University to discuss the Scientific Information Centers Branch's program and activities.

The Chief of the Branch and the Head of the Reproduction Population Research Information Center attended a two-day workshop in Library and Bibliographic Resources organized by the Carolina Population Center of the University of North Carolina. The meeting afforded an excellent opportunity for the Scientific Information Centers Branch to outline its work and plans, and conversely, to learn first-hand about the operations of other organizations.

The Assistant Chief of the Branch was a member of a discussion panel on Behavioral and Social Sciences and Information Services at the American Society for Information Services convention in New York. He also presented a paper on Complementary Services to Modern Data Archives at the meeting of the Council of Social Science Data Archives at the University of Pittsburgh.

Several members of the staff attended the MESH indexing course at the National Library of Medicine at various times during the year.

New Personnel:

Mr. Danny Towne, a Technical Information Specialist in Psychology, joined the Reproduction and Population Research Information Center August 27, 1967.

Mrs. Beulah Gyant, a Technical Information Specialist in Sociology, joined the staff of the Growth and Development Information Center October 10, 1967.

Mrs. Doris Stanley, a Computer Programmer, joined the Branch October 29, 1967.

Mrs. Judith Poindexter, an Input Operator, joined the Branch September 24, 1967.

Sidney Siegel, Ph.D., joined the Adult Development and Aging Information Center as a Technical Information Specialist January 14, 1968.

ADULT DEVELOPMENT AND AGING INFORMATION CENTER ACTIVITIES:

The staff of the Adult Development and Aging Information Center has completed the Base Vocabulary and is improving it by indexing articles from a group of core journals in Gerontology. The staff is also searching out periodicals in the fields of Biology (2,700 journals), Psychology (600 journals), Sociology (250 journals) and Related Sciences, to ascertain the priority level of the journals containing information on Gerontology. The priority rating will define the importance of journals for purchase by the Branch or use in the NIH Library and NLM.

An International Directory of Gerontology is being readied for publication with the target date of October 1968. Work on the Current Support of Research in Gerontology will be started during the next fiscal year.

GROWTH AND DEVELOPMENT INFORMATION CENTER ACTIVITIES:

The Growth and Development Information Center is currently being analyzed for practical divisioning. A series of meetings are being held between the NICHD Growth and Development Extramural Staff and the members of the Growth and Development Information Center staff to establish the direction of the Center.

REPRODUCTION AND POPULATION RESEARCH INFORMATION CENTERS ACTIVITIES:

During the year, the principal activities of the Center were the publication of "Current Support of Research in Reproduction and Population" and "A Bibliography of Gonadotropin Literature, 1966."

The "Current Support of Research in Reproduction and Population" contract negotiations with the Science Information Exchange involved a number of administrative and policy procedures which delayed the preparation of the report for many months.

"A Bibliography of Gonadotropin Literature, 1966" is unique in that it drew together information from four major indexing and abstracting sources. To enhance the usefulness of the compilation, two indexes were also prepared -- an author index and subject index. The KWIC indexing technique was used for generating the subject index.

At the request of the Branch Chief, the Reproduction and Population Research Information Center staff critically examined the usefulness and need to collect and bind reprints of papers published by the staff of NICHD. It was recommended that this work be undertaken and a modus operandi was proposed.

The staff will prepare in collaboration with the Science Information Exchange the next edition of the Current Survey of Reproduction and Population Research. Work will also be started on publication of a journal covering the literature of reproduction and population. Two alternatives will be examined. These are, collaborating with the NLM to publish a monthly citation journal or establishing an in-house operation to publish an abstract journal. It is planned to initiate publication of a journal on a regular basis by July 1, 1969.

TECHNICAL PUBLICATIONS OFFICE ACTIVITIES:

Publications: During fiscal year 1968, the Technical Pulbications staff edited, prepared the bibliography for and published "Patterns of Living and Housing of Middle-Aged and Older People," a conference; edited and published "Report of the Medical Exchange Mission to the USSR. Developmental Physiology and Pediatric Research."

Publications being processed: "The Reading Process," the proceedings of a conference sponsored by the Growth and Development Program is now being edited. Over 50% of the participants have returned their revisions which are being meshed as they are received. A tentative publication date of August 1, 1968 has been set.

Two conferences on "Low Birth Weight" are planned for the next fiscal year. The editor has designed covers for these books, compiled an extensive bibliography and has arranged for an index to be prepared.

Distribution of Publications: The Technical Publications Office has filled requests for copies of "Patterns of Living and Housing the Middle Aged and Older People" as well as 225 requests for past NICHD publications.

THE NICHD REFERENCE LIBRARY AND TRANSLATION SERVICE ACTIVITIES:

The Library's collection now contains 2,500 books, 228 scientific periodicals and 5,800 publications by grantees as well as college and university catalogs and similar reference material.

A total of 1,500 requests for books and periodicals were filled from the NICHD holds, the NIH Library holdings or by interlibrary loan. There were 350 new books ordered for the NICHD staff during the year. As the Library has an author and title catalog only, 1,975 of the Library of Congress cards ordered have been received and a subject file is being set up. The addition of a subject catalog and the assignment of call numbers to the books will improve the efficiency of the Library.

During the fiscal year, 483 pages of written translations from five (5) foreign languages were done by the translators as well as 50 pages of translations of letters.

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Office of Associate Director for Intramural Research Programs

The past year was the first full year of operation of the Intramural Research (IR) Programs which were established by the NICHD reorganization late in FY 67. Intramural Research Programs were given a firmly consolidated basis of operation on an independent footing during FY 68, a year marked by several significant developments.

A full time cadre for the Office of the Associate Director for Intramural Research was recruited, including an Administrative Officer. Procedures for handling the day to day business of the Intramural Research Programs were adopted, refined and placed in operation.

During April and May the first completed space in the new Gerontology Research Center (GRC) building at the Baltimore City Hospitals was turned over to NIH, and by mid-June the transfer of Intramural Research personnel into the new quarters had been completed. The building was dedicated on June 15, 1968. Since the Intramural Research Program must, in large part, structure its pattern of growth in accordance with the timetable on which space becomes available, the preponderance of Intramural Research resources for FY 68 were devoted to staffing and equipping the Gerontology Research Center. Recruitment was accelerated so that the Intramural Research portion of the building had approximately 40% of its ultimate staffing at the time of initial occupancy of the new building. As a first step toward meeting the needs of the Gerontology Research Center major outlays were made for new equipment. The needs of the Gerontology Research Center must be met before the new Intramural Research building at Bethesda and the installations in Puerto Rico require staffing and equipping. Toward the end of FY 68 the first collaborating units were assigned space, on an annually reviewed basis, in the Gerontology Research Center.

The Children's Diagnostic and Study Branch (CDSB), the Behavioral Biology Branch (BBB), and the Laboratory of Biomedical Sciences (LBS), which had previously cooperated in studies of mental retardation at the National Naval Medical Center (NNMC) were given separate resources and independent bases of operation during the past year. This has solved many of the problems which previously existed. However, several important problems remain. Early in FY 67 Dr. Loring Chapman, who had been Chief of Behavioral Biology Branch, accepted an academic post. Since then this Branch has been without full-time leadership, and has been held to four professionals. The Children's Diagnostic and Study Branch has only partially recruited the professional replacements necessary to serve some functions formerly filled by cooperating sister branches. One of the major problems of the Branch is an inadequate pool of patients. To remedy this deficiency we are exploring contract-supported activities at other institutions and the initiation of studies of infant populations at the National Naval Medical Center. The Laboratory of Biomedical Sciences has expanded significantly, has clearly defined its mission, and has established three new sections during the year. If recruiting efforts continue to be successful, this laboratory should achieve its short-term staffing goals during FY 69.

The Social and Behavioral Sciences Branch and the Developmental Biology Branch have not been notably expanded during the year. Space shortage continues to be a short-term problems for both branches, and the planned growth of these units will be coordinated with the timetable on which space becomes available.

During the FY 68 the Reproduction Research Branch made two attempts to establish a unit in a local teaching hospital with an obstetrical case-load which would be adequate to its clinical research needs. Neither of the possibilities which were explored could be activated. As a consequence, the Branch plans a significant expansion of its current basic research activities in the endocrinology of reproduction. This will occur during FY 69 as leased space becomes available to the Intramural Research Programs.

During the year, the Intramural Research Programs of NICHD sponsored 26 scientific seminars at Bethesda and a weekly seminar every Monday, September through May, at Baltimore. Regular monthly meetings of the Laboratory and Branch Chiefs were established. These, and other activities, have helped to define and promote the Intramural Research Programs of the Institute.

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Developmental Biology Branch

During the past year, there were four active foci of work within the Branch. Although a Chief has not yet been appointed for the Branch, its four section-equivalent units were able to operate quite effectively and independently. Leadership has been proved by the Scientific Director who has frequently been in touch with the investigators.

The Reproduction Anthropometry Section continues to gather accurate, extensive, longitudinal data on the human menstrual cycle and gestational interval. This massive, long-term undertaking is now coming to fruition with the publication of definitive information concerning temporal variations in menstrual intervals and menstrual and gestational durations throughout the entire life span. The data, gathered since 1934, may prove to be a unique resource because of the recently initiated wide-spread use of newer methods of contraception.

Dr. Michael Frank has continued his studies of the development of the immune system. During the year, he determined the number of active sites on the macroglobulin antibody (rabbit anti-Forssman). His exploration of the structure and mechanism of action of this molecular complex is particularly relevent to the mission of the Institute since macroglobulin is the first antibody to appear during development. It is the one upon which newborn infants principally depend for defense against infectious agents. At the end of this fiscal year, Dr. Frank will transfer his activities to NIAID.

Dr. William Greulich was able to establish effective contact with several institutions for the mentally retarded in the United States, and he has been able to procure from them a large number of hand and wrist radiographs which are currently under analysis. Particular attention is being given to the development of normal and abnormal skeletal features in children with Down's syndrome. It appears possible that certain skeletal abnormalities of the phalanges may be part of the syndrome. In addition, norms of skeletal development in the hand and wrist are being extended to Japanese who are citizens of the United States. In this way, it will be possible to check whether or not significant racial variation exists in these norms in racial populations living in the same environment.

The Section on Experimental Embryology has continued its study of the morphogenesis of the eye and of the inner ear. Activities during the past year have included: the modification of techniques to permit quantitative determination of ribonucleic acids in minute tissues such as the epithelium of the developing lens of the chick embryo; the use of previously developed infusion techniques in order to assess what permanent behavioral modifications might result from the exposure of embryos to catecholamines; and the use of microsurgical techniques that should make it possible to study the mechanisms underlying "sidedness" in the establishment of connections between two halves of the developing central nervous system. During the summer of calendar 1968, this Section will be moved to the Ophthalmology Branch, NINDB.

Serial No. HD-DB1
1. Developmental Biology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effects of pharmacologic agents and viruses on the developing ear and brain stem

Previous Serial Number: None

Principal Investigator: David Beal, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: This project sought to determine the effects on the developing auditory system of the mouse of chloroquine sulfate, kanomycin, and rubella virus administered to pregnant females. In addition, an attempt was made to assess damage to the inner ears of chick embryos, produced by introduction of propylthiouracil into the incubating egg. It was also planned to identify "critical" or sensitive periods during development (should such exist) for each of these agents.

Methods Employed: Among the techniques used were: determination of LD_{50}; administration of drugs and viruses to embryos beginning at different stages of development; use of the startle reflex to assess auditory function; histologic techniques and electron microscopy.

Major Findings: The LD_{50} for chloroquine given intraperitoneally to pregnant CBA-J mice was 110 mg/kg. Light microscopic studies suggested a lesion in the outer hair cells of the fetal cochlea. Initial startle reflext tests of hearing suggest a correlated loss in hearing ability.

Kanamycin sulfate injected into CBA-J mice on different days of gestation produced hair cell degeneration in the fetal cochlea.

Attenuated or non-attenuated rubella virus produce an antibody response in CBA-J mice. Three of a large progeny from infected females had eye and ear lesions and showed the presence of the virus. Twenty-four other progeny were without abnormalities and were free of virus.

Significance: Developmental pharmacological studies have not hitherto been numerous. These pilot studies yield some information on the specific types of lesions produced by different pharmacologic and viral agents. It is proposed to determine the times during their development at which specific target tissues are at hazard. More specifically, these studies seek to reveal the etiology of some of the types of ear disorder that, for want of information, can now only be classified as congenital.

Proposed Course of Project: This pilot study will be terminated on June 30, 1968 when the principal investigator will transfer to the Division of Indian Health. Prior to that time, he hopes to complete the study of the effects of propylthiouracil on the developing ear of the chick embryo, to reisolate the virus from the affected progeny of rubella infected mothers and to examine with the electron microscope the inner ear tissues of mice which had been drug-treated as embryos.

Honors and Awards: None

Publications: None

Serial No. HD-DB2
1. Developmental Biology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Lateral specificity in the neurogenesis of the optic system of the chick embryo

Previous Serial Number: None

Principal Investigator: Jane L. Coulombre, B.S.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: We seek to establish whether or not the axons of the retinal ganglion cells are specified as to side (right or left eye), and the extent to which this is part of the basis for establishment of highly regular patterns of decussation during embryonic development. It is planned to ask when such specificity appears during development, whether or not it is species-specific and whether it arises intrinsically or is dictated by tissues which surround the eye.

Methods Employed: Microsurgical techniques developed in this Section are being used to transplant the eyes of chick embryos from one side of the head to the other early in development. The independent variables are: host age, donor age, donor species, and the sidedness of the graft. The dependent variables deal with the pathways taken by axons growing out of the transplanted eye, and whether they reach the right or the left tectum. Silver impregnation techniques will be used to determine the course taken by the axons leaving the graft.

Major Findings: The surgical technique which will make this study possible has been developed. Preliminary results indicate that when either right or left eyes from three day donors are transplanted to the right orbital bed of three-day hosts, all their axons grow to the contralateral tectum.

Significance: In the development of neuronal circuity, connections are established not only among the several levels of the nervous system, but also from one side of the body to another in a complex but orderly pattern of cross-connections. We hope to shed light on when these cross-connections are established, whether the order in which they develop is obligatory or whether each step is independent, what properties of outgrowing axons and of the primitive nervous system through which they grow guide the fibers to establish appropriate connections, and to answer a number of related questions. Answers to these questions are essential to: a) any understanding of how information encoded at the genetic level achieves expression in the developed nervous system, and b) determining when during development and why one or another pathway or nucleus may be at hazard to insult introduced from without.

Proposed Course of Project: Now that the necessary surgical techniques have been developed, the questions outlined above will be systematically attacked.

Honors and Awards: None

Publications: None

Serial No. HD-DB3(c)
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Skeletal development of normal and abnormal children

Previous Serial Number:

Principal Investigator: William W. Greulich, Ph.D.

Other Investigators: None

Cooperating Units: Laconia State School, Laconia, New Hampshire
University of California Medical Center, Los Angeles, Cal.
University of Wisconsin

Man Years:

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: To analyse hand and wrist radiographs of patients with Down's Syndrome in order to compare them with normal and to detect skeletal correlates of this syndrome; and to determine the incidence of dysplastic middle fifth phalanx of the hand in American-born Japanese children.

Methods Employed: The study utilizes routinely obtained radiographs of the hand and wrist. These are analysed for normal and abnormal patterns of development of skeletal form and sequences of ossification.

Major Findings: In pursuing my interest in the skeletal development of normal and abnormal children, I have undertaken the following studies during the current fiscal year:

1. I made radiographs of the hands and wrists of all the patients with Down's Syndrome resident at the Laconia State School, Laconia, New Hampshire.

2. Through the generous cooperation of Dr. Stanley Wright, of the University of California Medical Center, Los Angeles, I obtained hand-radiographs of the patients with Down's Syndrome at the Pacific State Hospital, Pomona, California. These are patients on whom Dr. Wright and his associates had made chromosomal studies and on whom longitudinal anthropometric and other pertinent data are also available.

3. Through the kindness of Professor G. Lawrence Rarick, of the University of Wisconsin, I have obtained copies of hand-radiographs of children from two state institutions for the mentally retarded in Wisconsin on whom Professor Rarick and his associates have made other observations. This series is, so far as I know, unique in that it consists of radiographs that have been repeated on the same children at regular intervals over a period of years. The material thus makes it possible, in some cases, to follow the development of normal and abnormal skeletal features visible in the hand-film from their first appearances until they have attained their adult form.

The assembling of these data is now almost complete and awaits only the receipt of additional films from California and Wisconsin. A preliminary study of the material already received has been completed and a detailed study of all of it will begin as soon as all the material is at hand.

4. I have also completed a study on the hand-films of approximately 900 American-born Japanese children living in California, in order to determine the incidence of a dysplastic middle fifth phalanx of the hand, a congenital, hereditary defect which has been found to occur more frequently among Japanese, some American Indians, and other groups of more remote mongoloid ancestry than it does among the white population of this country. This has some pertinence for the study of the children with Down's Syndrome in which it appears that the incidence of the same defect is much higher than it is among the normal Caucasian populations from which these mentally defect children were derived.

Significance: Hand and wrist radiographs are widely used to assess the degree of maturity of children. These studies seek to: 1) test and extend the reliability of the criteria used in judging such radiographs by studying racial and regional variations, and 2) extend the usefulness of such radiographs to mental retardates such as those with Down's Syndrome by establishing correlative skeletal abnormalities.

Proposed Course of Project: I computed and have reported in preliminary form at the recent meeting of the American Association of Physical Anthropologists, in Detroit, the results of my study of the variability in the order of the first appearance of ossification in carpal centers as seen in the hand-film. Our findings bear on the question of the reliability of these ossification features as maturity indicators in the skeletal assessment of the hand-films of normal and abnormal children.

The report of our findings on the dysplastic middle phalanx of the fifth finger among the American-born Japanese children and the report on the significance of variation in the order of carpal ossification are now being prepared for publication. It is planned to submit both papers to the American Journal of Physical Anthropology within the next five months.

Honors and Awards: None

Publications: None

Serial No. HD-DB4
1. Developmental Biology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effects of drugs administered to chick embryos on post-hatching behavior

Previous Serial Number: None

Principal Investigator: Stephen W. Parker, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: The objective is to determine whether, and to what extent, catecholamines and substances which inhibit their production or release can effect permanent modification of post-hatching behavior when they are chronically administered to the chick embryo.

Methods Employed: Pharmacologic agents are introduced continuously into the incubating chick egg by a modification of the catheterization and continuous infusion technique previously developed in the Section on Experimental Embryology. The behavioral test currently in use measures the rate at which newly hatched chicks follow an object on which they are becoming imprinted.

Major Findings: The LD_{50} of epinephrine as a function of age is being determined. This will permit infusion of this substance at rates compatible with survival and yet offering a maximum chance that any behavioral effects will be revealed. Initial results indicate that chicks which were treated as embryos with epinephrine imprint more quickly (learn faster) than control chicks which had been given saline.

Significance: Among mammals, including human beings, drugs administered to the pregnant female often reach her embryo (s) or fetus (es) in effective concentrations. A good deal of attention has been given to the production of physical defects in such cases. Virtually nothing is known about the extent to which behavioral patterns may be permanently altered by prenatal exposure

to pharmacologic agents. This study seeks to implement the commitment of NICHD to Developmental Pharmacology by asking to what extent, and in what specific ways, behavior can be modified by such treatment.

Proposed Course of Project: Should the initial results be borne out the age of onset of treatment will be used as an independent variable to learn whether or not the effects produced by epinephrine are stage specific. Additional agents(Reserpine, Chlorpromazine) will be used. Behavioral tests other than imprinting will be employed. Endpoints other than behavioral ones (weight, adrenal histology, blood vessel diameter etc.) will be used. The initial phase of this work should be completed by June 30, 1969.

Honors and Awards: None

Publications: None

Serial No. HD-DB5
1. Developmental Biology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Lens fiber formation in-vitro and in-vivo

Previous Serial Number: None

Principal Investigator: Joram Piatigorsky, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: The purpose is to identify the molecular basis of embryonic determination which often preceeds the appearance of specific proteins or of visible cellular changes.

Methods Employed: Elongating lens epithelial cells from explants of six day chick embryos (Philpott and Coulombre, Exper. Cell. Res. 38: 635-644, 1965) are the objects of study. Histological, ultrastructural, autoradiographic and scintillation counting methods are being used to compare epithelial and fiber cells of the six day lens with the elongated, cultured epithelial cells.

Major Findings: 1. Explanted lens epithelial cells (initially 11 micra in height) begin to elongate immediately in culture and continue to do so linearly for at least 9 days at which time they are some 20 times (210 micra) longer than they were initially. 2. Lens epithelial, fiber and cultured epithelial cells incorporate tritiated uridine into 45s, 28s, 18s, 4s and heterogeneously sedimenting RNA. The percentage of label that sediments heterogeneously in a sucrose density gradient (relative to the amount of radioactivity attributable to RNA particles larger than 4s) is about 83%, 87% and 73% for the epithelial, fiber and cultured epithelial cells respectively. 3. "Pulse-chase" experiments with epithelial and fiber cells indicate that 28s, 18s and 4s RNA are relatively stable and that they accumulate while 45s and heterogeneously sedimenting RNA turn over at a faster rate. In 3 hours there is decay of almost all of the 45s RNA which had previously been labelled for one hour. In 3 hours there is a decrease in heterogeneously sedimenting RNA label from 87% to 55% for fiber

cells and from 83% to 40% fro epithelial cells (with respect to the radioactivity of RNA particles with s values greater than 4. 4. The present interpretation of these results is that epithelial cell elongation does not per se, involve any gross alteration of RNA anabolism or catabolism.

Significance: The general significance of this work is its promise to reveal a physical basis for the developmental phenomenon of determination. In a more specific way the work is characterizing the protein synthetic machinery that controls the synthesis of lens specific protein. This information can be applied to such diverse phenomena as lens growth and the development of lens cataracts. The Section also wishes to apply the results to an explanation of how lens size is coordinated with eye size during embryonic development.

Proposed Course of Project: Currently, the dynamics of DNA, RNA and the protein synthetic system are under study in order to determine how lens fiber formation occurs. Ultimately we hope to establish the nature of the program in epithelial cells that dictates lens fiber formation.

Honors and Awards: None

Publications: None

Serial No. HD-DB6
1. Developmental Biology Branch
2. Section on Experimental Embryology
3. Bethesda , Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Early morphogenesis of the cornea of the chick embryo

Previous Serial Number: None

Principal Investigator: Robert L. Trelstad, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.0
Professional: 1.0
Other: 0.0

Project Description:

Objectives: The Section seeks to determine the nature of the fibrous component of the presclera, the manner in which it is deposited and the contribution it makes to the collagenous architecture of the mature corneal stroma.

Methods Employed: Microsurgery,histochemistry (silver impregnation of the Golgi apparatus) and electron microscopy were used to study the developing cornea of the early chick embryo.

Major Findings: The Golgi apparatus, a cell organelle associated with secretory function, makes a transient move from the apical to the basal region of the innermost cells of the anterior corneal epithelium during corneal development. This transient alteration of cell polarity is correlated with the deposition of fibrillar lamellae in the underlying presclera. The geometry of these lamellae, and the orientation of fibrils within them foreshadow the architecture of the adult stroma, and presumably serves as a scaffolding for the development of the mature stroma.

Significance: These studies are casting light upon several important phenomena: 1) The role of epithelial sheets in the deposition of subepithelial plys of fibrils. Such plys may serve as miniature frameworks which become expanded by the polymerization upon them of collagen synthesized by fibroblasts. 2) The role of the Golgi apparatus in the synthesis of such plys requires attention. The developing cornea provides the best system known for the conduct of such a

study. 3) Corneal scarring following deep wounds may occur because the orderly deposition of fibrils depends on a prexisting scaffolding, the presclera. Since this scaffolding is normally synthesized only once during life and is thus not present to guide collagen deposition during the healing of wounds in the adult cornea.

Proposed Course of Project: The studies of cell polarity will be extended to such systems as the somite, limb bud and primitive streak to determine the contribution of polarity to early embryonic morphogenesis. Studies of the corneal system will be expanded to determine what factors determine the precise fibrillar orientation observed in the prescleral ply. One of the hypotheses that will be tested is that mechanical forces acting tangential to the corneal surface help determine extracellular macromolecular orientation in the presclera.

Honors and Awards: None

Publications: None

Serial No. HD-DB7
1. Developmental Biology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Status of protein synthesis in the fibers of the developing lens nucleus

Previous Serial Number: None

Principal Investigator: Keith M. Zinn, M.D.

Other Investigators: Calvin Hanna, Ph.D.

Cooperating Units: Department of Pharmacology, University of Arkansas School of Medicine, Little Rock, Arkansas

Man Years:

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description:

Objectives: The aim was a study of the protein synthesizing ability of lens fibers at different stages of embryonic development and a correlation of these findings with the ultrastructural observations of the lens fibers, and especially the ribosome population.

Methods Employed: Lens from chick embryos at 5, and 14 days of incubation and 8 days after hatching were studied: The techniques of autoradiography (double labelling), electron microscopy, sucrose density gradient separation and liquid scintillation counting were used. In-vitro and in-vivo isotope labelling was done with the following compounds: algal hydrolysate (C^{14}), uridine (H^3) and Leucine (H^3).

Major Findings: No protein synthesis takes place in the non-nucleated lens fibers found in the core of older lenses. (e.g. 8 days post-hatching). This correlates with the absence at this age of polyribosomes in this region of the lens, as revealed by electron microscopy and density gradient centrifugation of lens nucleus extracts.

Significance: Whether or not there is a long lived messenger RNA in the vertebrate lens, and for how long protein synthesis continues in the fibers of the lens nucleus after they have lost their cell nuclei, are subjects of

current debate. The present study indicates that protein synthesis and polyribosomes do not occur in the lens nucleus at least as early as 8 days after hatching. These findings have important implications for several facets of lens development, including the manner in which the lens grows.

Proposed Course of Project: This study is complete. It's substance will be reported in a scientific paper which is now in preparation.

Honors and Awards: None

Publications: None

Serial No. HD-DB8
1. Developmental Biology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Changes in corneal ultrastructure resulting from early lens removal in the developing chick embryo

Previous Serial Number: None

Principal Investigator: Keith M. Zinn, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.8
Others:	0.0

Project Description:

Objectives: To study the normal corneal development in the chick embryo as well as the aberrations in corneal development that result when the lens is removed from the eye early in development.

Methods Employed: Operations carried out at four days of incubation were as follows:

1. Lentectomy.
2. Lentectomy-control (lens removal from the eye with immediate reinsertion into its former location).
3. Normals (untreated).
4. Intubated eyes (introduction of capillary drainage tubes into wall of eyes at four days of incubation).
5. Intubated-control (introduction of solid glass rods into wall of four day eye).

The corneas were examined at various time intervals (from immediately post-operatively to thirteen days postoperatively) using the light and electron microscopes.

Major Findings: Several hours post-lentectomy a wave of mesenchyme tissue covers the posterior surface of the cornea to form a retrocorneal sheet of tissue. This sheet goes on to differentiate into sclera-like tissue. The cornea itself fails to grow in surface area and diameter. Untrastructurally

the anterior epithelium, basement membrane and the subepithelial zone look normal. No endothelium or Descemet's membrane is formed. Intubation does not affect the ultrastructure of the cornea.

Significance: This study was one part of an ongoing effort to demonstrate which steps in corneal development are under lens control and which are not. The present study suggests that the lens holds in check a disorderly invasion of limbic mesenchyme.

Proposed Course of Project: This project will be completed by June 30, 1968. The results are now being incorporated in a paper for publication in a scientific journal.

Honors and Awards: None

Publications: None

NICHD Annual Report
July 1, 1967 through June 30, 1968
Reproduction Anthropometry Section
Developmental Biology Branch

The long-term research program concerning menstrual and reproductive history, initiated by the Section chief in 1934 at the University of Minnesota and continuously maintained by him since then, continues to be the major research activity of this Section. A historical sketch of development of this undertaking was given in the annual report for last year. Program development anticipated at that time has proceeded as far as available funds have permitted. The scope of activities envisioned then was of considerable breadth, but lack of funds for certain new developments in data assembly has led to increased concentration by staff on completion of other phases. Intense exploitation of the great volume of data already on hand is proceeding along paths as planned.

Despite the heavy involvement for staff that analysis of our established data resources requires, it is regretted that expansion of the program to include the American Negro has had to be abandoned. A complete coverage of a geographically prescribed community of economically impoverished people, with advisory professional health services as part of a full supervision program, would have provided unprejudiced insights into population problems that must remain as an urgent need.

Interest of the senior staff in the temporal aspects of prenatal development and in the process of delivery of the live birth is also of long duration. Statistical analyses of extensive data from unusually reliable sources which became available to us many years ago led us to conclude then that errors in the date of LMP onset, as recorded routinely in hospital records, were not responsible for more than a relatively small part of the wide variation in the interval from LMP onset to birth. Uniquely accurate records of this interval provided by the menstrual history program have now confirmed the earlier conclusion. Considerable effort has been devoted this year to the preparation of papers for scientific journals in exposition of these facts. Reports on three different aspects of this problem have been completed; one is published, another is in press, and the third will have been presented orally before FY '68 closes. A fourth paper to conclude the series is under development.

A detailed account of progress in each of these activities follows in Project Reports HD-RP4(7), HD-RP5(7), and HD-RP9(8). Concentration in these activities has necessitated deferral to future years of research aiming to develop criteria of maturity at delivery of human and other primate live-born.

Serial No. HD-RP4(7)
1. Developmental Biology Branch
2. Reproduction Anthropometry Section
3. NIH, Bethesda, Md.

PHS - NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Quantitative Definition of the Human Menstrual Cycle

Previous Serial Number: SAME

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigators: Borghild G. Behn, Ph.D.
University of Minnesota School of Public Health

Cooperating Units: University of Minnesota Health Service
University of Minnesota School of Public Health
The Kimberly-Clark Corporation

Man Years:
Total: 3.4
Professional: 1.8
Other: 1.6

Project Description:

Objectives:

(a) To define the patterns of temporal variation in the human menstrual cycle and pregnancy experience throughout potential reproductive life.

(b) To define and trace to their origins any systematic differences revealed between communities of persons in their menstrual periodicities.

(c) To study the effects of use of contraceptive drugs and mechanical devices on the menstrual cycle and subsequent pregnancy experience.

Methods Employed:

Data assembly.

Menstrual histories, inclusive of reproductive and contraceptive data, continue to be assembled from the four sources which have now been established. An accounting of cases in these sources follows.

A. The total Minnesota series. Three distinct panels constitute this series: (a) the original group, enrolled almost entirely from the University student body in the period 1934-8; (b) daughters of the original panel members who have elected to join their mothers in this study; and (c) a new student panel enrolled in the period 1961-4 for comparative studies after a lapse of approximately 25 years. Of the original panel of very nearly 2300 members, 371 were still active in menstrual record keeping in calendar year 1967. Withdrawals because of hysterectomy or confirmed menopause will reduce the number of active cases by at least 38 in 1968. We anticipate that within the next few years practically all the original panel members will have completed menstrual life.

Over 450 daughters of original panel members are now active menstrual record keepers in the sibling panel; 13 were added in 1967, and 11 additions have already been made in 1968. This daughter series is not expected to increase much beyond 500, but the histories obtained from them should be adequate to enable numerical specification of (a) many mother-daughter relationships, and (b) adequate definition of secular trends in menstrual experience in the immediate post-menarche years.

The new student panel within the MS series, originally numbering 1367 enrollees, retains 935 active cases at the close of calendar year 1967. This panel, together with many members of the daughter panel, are now in the reproductive years; they provide nearly all of the information being received on oral contraceptives. These histories are invaluable for assaying the side effects of the pills used, for the records are freer from the influence of those factors of self-selection which might affect contraceptive reactions than is the case with records provided to the FDA by gynecologists and manufacturers.

B. The Alaskan Eskimo series. Enrollment of 650 Eskimo girls and women in six villages and the town of Bethel in the Yukon-Kuskokwim-delta area was completed in March of this year under reimbursable agreement with the Arctic Health Research Laboratory (AHRL). To these, 220 Eskimos at Pt. Barrow have now been added to strengthen our ability to detect any length-of-day effect on the menstrual cycle. These two panels constitute the Eskimo calendar (EC) series, each member of which is to be followed for three years. The special calendars for these indigenous peoples are designed in the conventional form of single months, and the completed months are collected by the nurses on their periodic visits. The results are proving to be highly satisfactory, less difficulty being experienced in Eskimo record keeping than was expected. Supervisory consultation with each panel member every two or three months is proving adequate; very few failures to record occur except during summer migrations to fishing camps when calendars tend to be mislaid temporarily.

C. The native Alaskan menarche series. A survey of all female students has been made in the residence schools of the Bureau of Indian Affairs at Wrangell, Mt. Edgecumbe, the Pribiloff Islands, in five villages in the Bethel area not included in the EC series, and in the towns of Nome and Unalakleet. Data on menarche and relevant associated information has been secured within these schools under nurse supervision in a census-type operation of complete coverage. This has yielded almost 900 records for an Eskimo menarche (EM) census which will be subjected to probit analysis to supplement the recorded date of menarche information arising in the EC series.

D. Caucasian calendar keepers in Alaska. The cohort of calendar record keepers among Caucasian teachers of the Anchorage Borough School District and some of their friends, initiated in mid-winter of 1967, yielded 238 responses and 150 enrollees. The low response rate (30 per cent) stimulated a second (and final) drive early this year to increase this number if possible. We now anticipate approximately 200 active calendar keepers in this Alaskan general (AG) series. The low enrollment rate (approximately 20 per cent), contrasting markedly with that of practically 100 per cent established in the EC series, in part reflects an unexpectedly high proportion of menopause and hysterectomy cases among the teacher group. Also, the use of contraceptive pills within this well-educated group almost negates enthusiasm for menstrual record keeping. Our studies with the Minnesota series shows that the pills are used by many for timing the menstrual periods as well as for contraceptive purposes. There is also the factor of a disinclination to commit oneself to record keeping on a very personal matter without any immediately recognizable compensatory gain. Although this AG series is very small relatively, it is anticipated that it will still serve a useful purpose as a contrast group with the EC series. Also, in comparisons with the MS series with which there is high comparability in economic and educational matters, it will prove valuable.

Data Processing:

Returns of menstrual records and medical reports from the total MS series for calendar year 1967 indicate that, of the total 4350 (approximately) first year enrollees, 1786 are still active as record keepers. These returns have been edited and coded in this office. Late returns will probably increase their number to about 1800 by the end of June; an addition of about 30 cases may occur after that month but within the current calendar year.

The University of Minnesota, in addition to its sponsorship of data collection for this study, provides all computer services for storage, tabulation, and large-scale statistical processing of the accumulated data through contractual agreement with NIH. Formulation of specifications for this extra-mural work has comprised a substantial segment of the research activity this year for the Section Chief and Statistician.

Coding of all the data accumulated within the project through calendar year 1966 was completed early in the present fiscal year when data from the new student panel was added to the master tape files. Consistency checking by computer of the total input to those files, involving progressive development of an unusually complex program, is now completed.

Major Findings:

Conclusions presented in the last annual report concerning menstrual interval (onset to onset) from the 25,825 person-years of interval experience through calendar year 1961, have been tested against the comprehensive recoding by staff of this Section of all information of record through 1966. The excellent agreement found gives assurance that the earlier coding by a clerical staff outside NIH for the one variable studied at that time was completely satisfactory. From the viewpoint of basic biology, menstrual interval has now been determined without question as being characteristic of the individual woman and not of the sex as a whole. The 28-day cycle is a statistical value approached by many, but experienced by few. The term "regular" seems to be synonymous with "normal" when women report medically on variation in their cycle lengths. Through middle life (20-40 years of age), after the transition from post-menarchial experience has been completed, the range of variation for the central 90 per cent of intervals decreases from two weeks at the younger age to 10 days just preceding the pre-menopausal transition.

Current interest in side effects of the oral contraceptives has led us into detailed desk analyses of our records through 1966. Somewhat over 750 presently active panel members have used these control drugs at some time during the course of their recording of menstrual history. Approximately seven per cent have made notations of side effects, these varying from the common minor discomfort symptoms to the several acute conditions which have been given prominence in the popular magazine articles directed to women. Concern is justified by confirmation of the types of serious sequelae which our data reveal, including the death of one young physician after three years usage of oral contraceptives. Thrombolitic involvements of the eye vary in their degrees of impairment of vision, including temporary blindness and beyond in one case. Symptoms of developed psychoses seem more common than has received attention; attempted suicides are recorded. Recovery is slow following cessation of pill ingestion. Many physicians ignore the pill as a potential cause of patient distress. When the records of calendar year 1967 are incorporated, our data on side effects of oral contraceptives should be extensive enough to justify their publication as a statistical summary. Records from a population sample free from biased selection with respect to this very important variable may not be available elsewhere.

Preliminary study has likewise been made of the effect of a course of contraceptive drugs on subsequent menstrual cyclicity and pregnancy during abstinence from such control procedures. The findings are again tentative; our data prior to calendar year 1967, which include the combination of experience before, during, and after oral contraceptive use, are presently limited to 111 cases. The interval between menstrual onsets so far appears to be lengthened on the average about 1.5 days, and the duration of flow is decreased by almost one-half day. The variability both of menstrual interval and duration of flow are decreased about 25 per cent on the average. In the individual case, however, the tendency to return to the pattern of pre-control cyclicity is sufficiently strong that the average change is only of value in statistical assessments.

A larger sample (N=354) was available for advance study of the effect of control drugs in altering prior uncontrolled experience. A surprising result here is the persistence of considerable variability in both interval and duration when control drugs are used. Although an average reduction in menstrual interval of five days is recorded, the control is by no means complete in the individual case; intra-person intervals under control drugs still varied over a range of seven days. This finding probably reflects the growing practice of women to use the pills not only for contraception but also for regulating menstrual periods so that they occur at more convenient times.

Differences between the brands of oral contraceptives in their effects on the cycle seem trivial so far, although at present there are indications that the sequential type may prove to be a better stimulant of subsequent fertility when control is ceased.

Significance to Bio-medical Research and the Program of the Institute:

The current emphasis throughout most of the world on the need for birth control measures as a deterrent to the "population explosion" gives considerable significance to this program for its impact on bio-medical issues of the day. The excitement of discovery of fairly simple contraceptive measures, and promotion of their use by governments as well as private organizations, has focused attention on hoped-for results rather than on the issues of more substantive character. This program's emphasis is on establishment of fact in the domain of basic biology; campaigns to push ahead the greater use of controls on creation, as they generate the watch-words of the day, overlook the possibility that current synthetic controls are not yet proven to be medically safe for all, nor economically within the reach of all. Body balances with respect to hormonal controls are often delicate and may be as individual as we have shown the menstrual cycle to be.

Proposed Course:

Projected analyses within this program for FY '69 will be concerned primarily with the topics listed below if present priorities remain undisturbed.

(a) Establishment of the form of frequency distribution of menstrual intervals, within all persons collectively as a generalization, from menarche to menopause by single years of age, with provision of probabilities of occurrence of the intervals between menstrual onsets. This will end current dependence on normal curve approximations which are obviously too gross to be useful.

(b) Full specification of the frequency distribution of duration of menstrual flow, in equivalent detail to that given for the onset-to-onset interval.

(c) Study of the interval from cessation of flow to the next onset to determine which interval has more stability and hence the greater usefulness.

(d) Survey of the side-effects experienced by users of oral contraceptives within this program.

(e) Determination whether season of the year has any effect on menstrual phenomena.

(f) Specification of requirements for computer output to facilitate subsequent analyses.

Data gathering activities through a long term of years have been essential to this program. Continuance of this phase of the total operation will not be extended beyond the point at which the objectives in statistical analysis can be reached. Phasing-out of data assembly will begin in calendar year 1970 and be completed at close of the following year. At that time the panels within the MS series will have provided adequate data for definitive solution of the remaining problems in the post-menarche and pre-menopausal phases of menstrual experience. The pattern of performance then established will cover the complete span of menstrual life for a large group of Caucasians. This pattern will serve as the reference standard for comparison with other ethnic groups. It is expected that professional staff of the Section will be deeply involved during calendar year 1972 with preparation of final reports based on intensive statistical analysis maintained throughout the interim years.

Publications:

Treloar, Alan E., Boynton, Ruth E., Behn, Borghild G., and Brown, Byron W. Jr.: Variation of the human menstrual cycle throughout reproductive life. International J. Fertility. 12: 77-126, Jan.-Mar. (#1, part ?), 1967.

Serial No. HD-RP5(7)
1. Developmental Biology Branch
2. Reproduction Anthropometry Section
3. NIH, Bethesda, Md.

PHS - NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Time Relationships Involved in Human Gestation

Previous Serial Number: SAME

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigators: Borghild G. Behn, Ph.D.

Cooperating Unit: Perinatal Research Branch, NINDB

Man Years:
Total: 1.5
Professional: 1.3
Other: 0.2

Project Description:

Objectives:

(a) Quantitative specification of the human gestational interval (from onset of LMP to delivery) with respect to its variation patterns and probabilities for live births.

(b) Determination of any ethnic group differences in this variable.

(c) Evaluation of the effects of error in recall of LMP in private and general clinic cases, using precise data from the menstrual history program as the standard of reference.

Methods Employed:

Data have been assembled by senior professional personnel of the Section from five unusually reliable sources, differing in time period and geographically. These have been studied statistically in uniform analyses with respect to typical value and dispersion. Graphical methods permitting highly accurate determinations of probabilities have been used also to display group differences.

Major Findings:

The interval from onset of LMP to birth of the live child has been defined precisely for the first time. Records of pregnancies provided by the menstrual history program wherein LMP is recorded currently, yield precise data on gestational intervals. Error of recollection of LMP affects all other records to an unknown degree.

The range of variation in gestational interval for 2083 cases in the error-free MH data is found to be from six months to one year approximately. This range agrees very well indeed with that derived from 882 records from Dr. Fred Adair's private practice in Minneapolis for 1927-30, 8987 cases from the Sloane Hospital in New York City for 1890-1920, 4950 cases from the Minneapolis General Hospital for 1930-33, and 9055 cases provided by the 13 cooperating hospitals within the NINDB perinatal research through the period 1959-63. Only white live births were used in these studies, all data being carefully reviewed for completeness and dependability. It is thus established that errors of recollection in LMP date are of trivial consequence in the extremely short and long gestational intervals.

Within these limits, the pattern and amount of variation differs somewhat between the two major groupings of data sources - private case records (MH and Adair) and the hospital general clinics. These differences suggest that errors of recollection are probably responsible for an increased standard deviation for general clinic data, reflected in a more "flat-topped" distribution for them in comparison with precise records. In extreme deviation cases this error becomes relatively trivial but, of course, does not disappear.

Data provided by the cooperative study of NINDB have been used to show that highly significant differences exist between the ethnic groups designated as white, Negro and Puerto Rican in that program. These differences involve typical values, dispersions and frequency distribution form. Hospital differentiation as well as interaction between hospital and ethnic group factors have been demonstrated, but they are of lower significance than that between the racial (or ethnic) groupings.

The extent of variation confirmed in the error-free records of the MH program, and repeated in all other series of gestational interval data, is logically assignable to factors revealed only in the MH records. In large part the gestational intervals less than 8 lunar months may be due to post-pregnancy cyclic bleedings stimulating menstruation. For the very long gestational intervals it is demonstrable that very early and unrecognized pregnancy losses, without occurrence of a menstruation before the stable pregnancy is achieved, can be causal.

Significance to Bio-medical Research and the Program of the Institute:

The current concept that the rate of intra-uterine development of a product of pregnancy is fairly uniform for all gravida must be subjected to more careful scrutiny. The great post-partum variation in rate of development of the newborn, which is so readily observable, most probably holds also for intra-uterine development. And whatever is true with respect to physical development may have its parallel in the factors influencing mental development. To assess the physical growth factors with greater precision, it will be necessary first to find reliable criteria of the beginning in time of the pregnant state. Differentiation of cyclic vaginal bleedings pre- and post- initiation of pregnancy would do much to remove the ambiguity involved in the current practice in vital statistics of using the term "weeks of gestation" for the interval from LMP to birth. The concept that maternal menstruation cannot occur after establishment of her pregnancy needs critical reconsideration from a practical point of view. Acceptance of anovulatory menstruations requires modification of deterministic theory; post-pregnancy cyclic bleedings may also deserve reconsideration that would shift theory more realistically to a probabilistic basis.

Proposed Course:

Much detail is available in the clinical data on gestational interval being generated by the menstrual history program. The study of such information for abortions in particular, and for stillbirths, will be advanced as rapidly as possible in the immediate future. Relationships between gestational interval and menstrual interval, each as affected by age, will be examined fully. Designs for collecting pertinent information from new sources can be formulated better in terms of insight more explicitly defined.

It is planned that vital statistics data will be explored in the near future to study the effect that change of the question on birth certificates concerning LMP has had on gestational interval determinations.

Publications:

Treloar, Alan E., Behn, Borghild G., and Cowan, Donald W.: Analysis of Gestational Interval. Am. J. Ob. Gyn. 99: 34-45, September 1967.

Serial No. HD-RP9(8)
1. Developmental Biology Branch
2. Reproduction Anthropometry Section
3. NIH, Bethesda, Md.

PHS - NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Relationships Between Pelvic Measurements and Duration of Labor in Spontaneous Deliveries, by Age of Gravida and Birth Order

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigators: Borghild G. Behn, Ph.D.

Man Years:
Total: 0.1
Professional: 0.1
Other: nil

Project Description:

Objectives:

(a) To determine the influence of age of the gravida, and the birth order and sex of the child, on the duration of labor in spontaneous deliveries.

(b) To evaluate the influence of size of pelvis on duration of labor in unassisted births.

Methods Employed:

Case records for all spontaneous deliveries in the Obstetrics Department of Minneapolis General Hospital for the period from 1 January 1930 to mid-year 1933 were abstracted with respect to 38 variables concerning both mother and child for 4705 cases having reasonably complete pelvimetry data. Only minimal use of instruments was permitted in the service during the period of study, and no drugs influencing labor were allowed. These data have been analyzed statistically for duration of labor (three stages) within birth order and maternal age classes. Analyses of relationships between pelvic measurements and duration of labor were made collectively for all maternal ages and birth orders; a breakdown into finer classes would have reduced the frequencies in correlation surfaces to numbers too small to serve useful purposes.

Major Findings:

Duration of spontaneous labor has long been recognized as a highly variable quantity. Our frequency distribution studies have not only defined the pattern of variation as a typically smooth and highly skewed distribution, but also as one changing in both typical value and degree of skewness with birth order. Duration of labor does not vary with age except as a correlate of birth order. The changes are most marked from the first to the second birth order, in the direction of a lessened skewness. There is a resultant reduction in median value from 13.92 hours to 8.63 hours. The shift in form and typical value continues as a decelerating trend through the third delivery to the fourth plus fifth deliveries, which were united for lack of adequate differentiation, before the trend is reversed. The median duration of labor for spontaneous deliveries reached a minimum of 7.45 hours for birth orders (4 + 5), returning to 8.62 hours for the 345 cases collectively with birth orders extending from eight to 16. Sex of the child influences duration of labor, male deliveries averaging 32 minutes longer than female deliveries.

Correlation studies focused on the relationship of pelvic dimensions to duration of labor yielded, in the main, negative results. Only two of the nine manual measurements showed any relationship to duration of labor; in those two the correlation was so small that it had no practical value. The value of pelvimetry for those deliveries that are completed spontaneously does not extend to predictive capacity concerning the length of time involved in labor.

Significance to Biomedical Research and the Program of the Institute:

This study provides the necessary basis for evaluation of the effects of oxytocic drugs in the reduction of duration of labor. Any such evaluations should recognize that ethnic factors may be influential on duration of labor, and therefore a return to the same general type of population should be made in assembling new data. Since oxytocic drugs are now in common use, it is difficult to establish adequately comparative groups within any one hospital without very careful design to assure unbiased allocation of the drugs in case management. The analyses reported herein may therefore attain their major value as a definitive description of time relationships with respect to a variable in basic biology which can no longer be studied adequately, since drug usage is now so widely ranging.

Proposed Course:

Investigation of interactions between sex, birth order, and maternal age with duration of labor for the cases under study will be completed before publication of these findings. It is anticipated that this project will then be terminated, the acquisition of new data for comparative analyses on related issues being left to others.

Serial No. HD-RP8(7)
1. Developmental Biology Branch
2. Bethesda, Maryland

ANNUAL REPORT
of
Michael M. Frank, Medical Officer
July 1, 1967 through June 20, 1968

Project Title: Structure of Macroglobulin Antibody and its Mechanism of Biological Activity

Previous Serial Number: HD-RP8(7)

Principal Investigator: Michael M. Frank, M.D.

Other Investigators: Dr. J. H. Humphrey
Dr. T. Borsos
Dr. H. J. Rapp
Dr. A. Percy
Dr. R. Mage
Dr. R. Asofsky

Cooperating Units: Immunology Section; Biology Branch, NCI; Germfree Laboratory, NIAID: Laboratory of Immunology, NIAID: National Institute for Medical Research, Mill Hill, London, England

Man Years

Total: 2
Professional: 1
Other: 0

Project Description

a. Objective

To study many aspects of the structure of rabbit anti-Forssman macroglobulin antibody. To determine the size of the subunits, the number of antigen binding sites, the chemical nature of the antigen binding sites, the chemical nature of the protein chains, allotypic specificity of the protein chains and details of synthesis of the protein chains.

b. Methods Employed

Purification of macroglobulin antibody by dissociation from antigen either as intact molecules or as subunits, radiolabelling with I-125 or with C-14, separation of chains in the ultra-centrifuge and by Sephadex chromotography, quantitative complement fixation titrations and complement dependent assay procedures, extraction and chromotography of antigen active material, incorporation of radioactive amino acids into protein in in vitro antibody synthesis studies.

c. Major Findings

Work on the basic structure and number of sites on the macroglobulin antibody was completed. As indicated last year, a new basic subunit of macroglobulin antibody consisting of one H and one L protein chain pair was discovered. It was found that one-half of the protein chain pairs in the molecules recognize antigen and one-half do not. This work was extended and completed. We find that the antigenic composition of the chains within that half of the molecule that recognizes antigen is the same as that which does not recognize antigen. Anti-Forssman antibody has been prepared in rabbits known to be heterozygous for various allotypic markers on the protein chains. Studies are in progress on the allotypic composition of IgM molecules. Thus far in very preliminary data no examples of individual antibody molecules with more than one allotypic marker has been found. Electrophoretic techniques are being used to attempt to separate the various protein chains which recognize antigen from those that do not. Attempts are being made to inhibit the antibody activity of the IgM with a large series of sugars and mucopolysaccharide analogs of Forssman antigen with known structure. Thus far no low molecular weight potent inhibitors have been found. Studies on the synthesis of this macroglobulin antibody are underway and in vitro system capable of producing radio-labelled immunoglobulins has been set up. It has been possible to isolate large numbers of anti-Forssman macroglobulin producing cells from the peripheral blood of rabbits. It is hoped to separate the IgG and IgM antibody producers.

Complement fixation by the IgM is being studied. Polyvalent IgM with broken disulfide linkages no longer fixes $C^1$1. It has been possible to prepare intermediates in the sequence of reactions which take place when antibody complex with cellular antigens are allowed to react with some of the component of complement. It has been possible to remove the IgM antibody after the attachment of complement and examine the fragments for the presence of complement proteins attached to the protein chains. Evidence thus far indicates that IgM antibodies become covalently linked with complement only when they are present in large numbers on cell surfaces. Preliminary data suggests that $C^1$4, the second complement component in the sequence of reactions leading to cell death, remains attached to cell surface when IgM used to make the active antibody site is removed. These studies are being extended at present.

d. Significance to Bio-medical Research

Macroglobulin antibody is the first class of antibody to appear in both phylogenetic and ontogenetic development. Infants synthesize macroglobulin during the period when their capacity to synthesize IgG is limited and their major protection against infection resides in the IgM system. There is no instance of a healthy child having defective macroglobulin antibody synthesis. These studies are directed at the finding the importance of macroglobulin antibody and determining its mechanism of action in a large number of immune systems.

e. Proposed Course

The work is to be extended to the study of other macroglobulin antibodies. The present problems will be investigated as will be the biologic importance of macroglobulin antibodies.

f. Honors

Election into the Association of American Immunologists, April 1968.

g. Publications

The Sub-Units of Rabbit Anti-Forssman Macroglobulin Antibody by M. M. Frank and J. H. Humphrey, Journal of Experimental Medicine, in press.

Reduction Fragments of Macroglobulin Antibody, M. M. Frank and J. H. Humphrey, in preparation for Immunology.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968
REPRODUCTION RESEARCH BRANCH

SUMMARY

The Reproduction Research Branch was formally organized about 11 months ago. The biological program had been functioning independently under the able direction of Dr. William W. Tullner. This biological unit has been kept intact as the Endocrinology Section, continuing under Dr. Tullner's direction. The Office of the Branch Chief functions essentially as a Clinical Studies Section of the Branch.

Since the initiation of the Reproduction Research Branch no new physical facilities have been provided for its use, and an administrative half-module in Building 31 previously assigned to the Branch Chief has been relinquished in order to facilitate the conduct of the Branch's program in Building 10.

In view of the demonstrated unsuitability of previously proposed contract arrangements for the clinical program of the Branch in either of the two locally competing hospitals, the clinical activities of the Branch have thus far been entirely dependent upon patient materials made available from other Institutes or from neighboring hospitals.

The already over-burdened laboratory facilities of the Endocrinology Section have been pressed into use in order to permit the initiation of the new studies which are detailed in the attached project reports. However, it has not proven practicable to recruit additional senior personnel pending the availability of additional space for their actual occupancy. The status of the Reproduction Research Branch with respect to personnel and space is detailed in my memorandum dated March 27, 1968. (copy attached)

The proposed and current research activities of the Reproduction Research Branch since its inception are outlined in the attached project reports. This program entails coordinated clinical and laboratory investigations of the reproductive process in man and animals. Whereas the initial emphasis is on the endocrinological phases of reproduction, it is expected that nutritional, genetic, and behavioral studies will be added as facilities may permit. The substantive content of these proposed studies has been set forth in several earlier memoranda, particularly that of January 15, 1968.

Notwithstanding space limitations, the Branch has substantially extended the area of its clinical and animal studies. Especially noteworthy are the primate studies for which a very limited but smoothly functioning facility has been effectively utilized. With increasing emphasis on primate work in

relation to clinical problems in reproduction this restricted resource presents a substantial limitation for our efforts.

The studies on reproductive failure in senile rabbits provide an animal model for the study of the endocrine and cytogenetic factors involved in the increased incidence of congenital abnormalities in older mothers. Since such retired breeding animals are a constant product of the Animal Production Unit, their further availability for study is assured.

The almost overwhelming issue of the immediate and long-term effects of the newer contraceptive agents now employed by millions of women presents a clinical as well as an epidemiological challenge for further study. Our participation in the projection and formulation of these studies by the Institute as well as our own detailed clinical studies constitute a major element in this Branch's interests.

Another major issue in reproduction research is the urgent need to extend behavioral studies at both a basic and clinical level. We regard our initial efforts in studying the effects of psychotropic drugs on steroid-hormone-induced copulatory behavior as an example of the type of experimental activity necessary to bridge the wide gap between biological observations and applied behavioral studies in this vital area. Further primate studies on sex-attraction, individual and group sex behavior, and the behavioral aspects of pubescence as modified by hormonal means have a high priority in our projected program.

Contraceptive development can be largely involved with the routine screening of minor variations in classes of chemicals or devices already found to be effective but not ideal. Alternatively, such development may be directed toward the identification of mechanisms yet to be identified through basic physiological research. Without neglecting the fruitful opportunities offered by the first of these approaches as exemplified in our studies of zygototoxic agents, we propose to emphasize physiological studies in both the male and female.

On the whole, one can realistically expect that a well rounded program of reproduction research will be evolved here at the National Institute of Child Health and Human Development once the requisite clinical and laboratory resources can be specifically allocated. Quite obviously, if the mission of NICHD's Intramural Program in this area is to develop there will be required adequate facilities for (a) domicilary care of women during all phases of pregnancy (b) intrapartum and post-partum care appropriately coordinated both with pernatal and post-natal observations in the mother and new-born and with subsequent observations on pediatric growth and development.

Meanwhile inquiries from highly qualified senior personnel increase daily particularly in view of some of the recent contraction in project-grant support. One can anticipate that the recruitment problem can be met once adequate space is made available.

May I take this occasion to express the warm appreciation of the staff of the Reproduction Research Branch for the unfailing support extended to us despite the limited physical resources available to the National Institute of Child Health and Human Development.

Serial No HD-RP 1
1. Reproduction Research Branch
2. Section on Endocrinology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Physiological and Pharmacological Studies on Reproductive Processes in Laboratory Primates.

Previous Serial No.: HD-RP1

Principal Investigators: Dr. Roy Hertz, Dr. William Tullner

Other Investigators: Mr. Bill Hopper, Dr. Griff T. Ross, Dr. John T. Wilson, Mr. Philip Rayford, Dr. Clinton W. Gray, Dr. John Lewis, Dr. Willis Brown

Cooperating Units: Endocrinology Branch and Surgery Branch, NCI, National Zoological Park, Smithsonian Institution

Man Years:

Total: 6
Professional: 2
Others: 4

Project Description:

Objectives:

1. To determine patterns of gonadotropic and steroid hormone production during pregnancy and the menstrual cycle of primates (rhesus monkeys, baboons, gorilla) in order to predict accurately such reproductive events such as ovulation, implantation and fetal loss.

2. To gain basic information on interrelationships of the anterior pituitary-adrenal-reproductive system under conditions of normal and abnormal hormonal stimulation.

3. To determine biological and immunological relationships between chorionic gonadotropins of man and subhuman primates (rhesus monkey, baboon).

4. To develop methods for semen collection, preservation and artificial insemination in rhesus monkeys.

5. To study the development of hepatic microsomal drug metabolism in postnatal and pubertal rhesus monkeys.

Methods Employed:

1. Monkeys are housed under carefully controlled conditions of temperature, lighting, and maintained on a standard diet.

2. Biochemical methods (using thin-layer and gas chromatography) have been developed for determining microgram quantities of steroid hormones.

3. Quantitative measurements of chorionic gonadatropins are based on standard bioassay procedures with radioimmunoassay methods in the preliminary stage of development.

4. Standard methods for study of drug metabolizing enzyme systems are used.

5. Semen is collected by electroejaculation using either rectal probe or phallus stimulation methods. Semen is preserved by freezing in liquid nitrogen.

6. Human choriocarcinoma tissue is recovered from the hamster cheek-pouch and transplanted to the monkey brain.

7. Surgical manipulations of the endocrine system are carried out through ovariectomy, adrenolectomy, and hypophysectomy.

Major Findings:

A. Physiological and pharmacological alterations in estrogen excretion of subhuman primates.

In continuing studies on the effects of anterior pituitary hormones on the steroid biosynthetic pathways of the adrenal cortex, administration of adrenocorticotropin (ACTH) produced a significant urinary excretion of estrone in ovariectomized female rhesus monkeys (Macaca mulatta).

Although lactation was present in these animals, it was not seen in ovariectomized-adrenalectomized monkeys. Likewise, estrone was not found in the urine of the latter. This finding points to the adrenals as the source of estrone or its precursors since estrogen is a prerequisite for lactation (Publication No. 1). These findings confirm our previous demonstration of estrogen-stimulation of the endometrium in castrated female monkeys following treatment with pharmacologic doses of ACTH.

The female sexual cycle of the rhesus monkey is a menstrual cycle of 28-30 days. Precise chemical measurements of urinary estrogen excretion were made throughout this cycle in a series of adult monkeys preliminary to correlating the stage of ovarian follicular development with urinary estrogen levels.

Estrone was the principal estrogen found with estradiol and estriol being excreted in smaller amounts. Estrone levels attained a sharp peak near mid-cycle with values approximately 7 times greater than the lowest levels in the cycle. The peak of estrone excretion corresponded closely to the predicted time of ovulation.

Chemical methods have been developed for the isolation and estimation of estrogens in placental tissues collected at intervals throughout pregnancy in the baboon (Papio anubis), Preliminary results show that estradiol-17 β and estrone were present. Estriol was not found.

In collaboration with the National Zoological Park, urines were collected throughout pregnancy in a gorilla. Urinary estrogen excretion was followed weekly from the 9th week of pregnancy to the post-partum period. Estrone, estriol and estradiol-17 β levels increased throughout pregnancy. As in humans, estriol was the characteristic hormone of late pregnancy.

B. Chorionic Gonadatropins

Investigations of primate placental gonadotropins have been continued. Improved procedures for extraction and concentration of urinary gonadotropin permitted immunization of rabbits with rhesus monkey chorionic gonadotropin (MCG). A potent rabbit antiserum to MCG was induced. This antiserum was capable of neutralizing human chorionic gonadotropin (HCG) in the mouse uterine weight assay. However, this antiserum was far more active in neutralizing the effects of its own antigen(MCG). Neutralization of MCG by a rabbit antiserum to HCG required twice the concentration of antiserum than that needed to neutralize an equipotent preparation of HCG. Bioassays of MCG and HCG in parallel, using mouse uterus weight and rat ventral prostate weight as end points, had shown a similarity in dose-response curves. In the biological systems used, therefore, both human and simian choronic gonadotropins evoked similar responses.

In the radioimmunoassay system for HCG which was developed by Drs.Griff Ross and William Odell, we had noted a lack of cross-reactivity when monkey chorionic gonadotropin was tested. However, with a new rabbit antiserum to HCG, there was a definite cross reaction with MCG both from urine and in serum.

Chorionic gonadotropin (MCG) excretion during early pregnancy was determined by bioassay in 2 series of pregnant rhesus monkeys. Although MCG was found as early as the 13th day in some monkeys, the hormone was not demonstrable in others until the 18th or 20th day of gestation (Publication No. 2).

In continued studies on pregnancy hormones of baboons(in collaboration with Dr. A. Hendrickx of the Southwest Research Foundation), serum chorionic gonadotropin was demonstrable as early as the 15th day of gestation and was no longer found after 42 days. The temporal aspects of chorionic gonadotropic hormone production in baboons (Papio anubis) and the rhesus monkey are quite similar. This increases the utility of the baboon for investigation of primate reproductive physiology.

C. Electroejaculation and semen production.

Methods for electroejaculation and semen storage have been developed. Electroejaculation of a healthy male breeder monkey over a period of 16 months did not provide evidence of aspermia in laboratory monkeys as has been reported for free-ranging monkeys in Puerto Rico.

A modified egg yolk, citrate, glycerine semen-extender appears to give maximum sperm viability, with survival in some samples after 14 months preservation in liquid nitrogen.

D. Liver Drug Metabolism.

Studies were continued on the development of hepatic enzyme activity in the postnatal period of the rhesus monkey. Limited findings indicate that the activity of the liver enzyme system which metabolizes several drugs in the young monkey increases at a much slower rate than that found for several rodents (rats, guinea pigs, rabbits).

E. Human Choriocarcinoma Transplants

Human Choriocarcinoma tissue which has been carried in serial passage in the hamster cheek-pouch for the past 8 years has been successfully transplanted and grows luxuriously in the brain of the untreated monkey, M. mulatta. Previous attempts to transplant this tissue to the uterus or lung of the monkey have failed repeatedly. The tumor developing in the monkey brain serves as an excellent experimental equivalent of the patient suffering from brain metastases.

F. Experimental Ovarian Dysfunction.

Subtotal ovariectomy of the monkey involving the removal of one ovary and 90% of the opposite ovary leads to continued but irregular menstruation. Histological study of the small but functional ovarian rest indicates a strong tendency to thecal luteinization in the follicles seen. This experimental picture simulates in some features the Stein-Leventhal syndrome in women.

Proposed Course of Project:

One of the principal aims in this project was development of sensitive radio-immunoassay methods, not only for monkey chorionic gonadotropin but also for pituitary gonadotropins. With the finding that MCG cross-reacts with an antiserum to HCG, we are able to proceed with measurements of chorionic gonadotropin which can be correlated with steroid hormone secretion in efforts to determine the role, of chorionic gonadotropin in primate pregnancy.

Currently, estrogen excretion levels in the rhesus monkey are being compared with sequential observations(laparotomy) on time of ovulation.

Studies on hepatic microsomal drug metabolism will be terminated in June, 1968, when Dr. John Wilson will leave NIH.

Current investigations on induction and regulation of maternal placental development include studies on the effects of a plastic intrauterine contraceptive device on decidual formation in the rhesus monkey uterus. Further characterization of the syndrome following sub-total ovariectomy is planned. We anticipate continuing the remaining studies along the lines indicated.

Significance to Bio-Medical Research and the Program of the Institute:

Subhuman primates such as those available in our breeding colony of rhesus monkeys provide excellent material for investigating problems in reproductive physiology, particularly the female with a menstrual cycle and reproductive system similarities to those found in women. Systematic examination of the gonadal and placental hormones combined with morphological studies of the ovaries and uterus present experimental means for understanding and regulating the function of thses and related endocrine tissues.

Improved procedures for collection, preservation and storage of semen will enable us to produce timed pregnancies as well as study tubal transport of sperm.

Honors and Awards - Dr. William W. Tullner

1. Invited to participate in panel on "Artifical Insemination in Primates" Annual Meeting of American Association for Laboratory Animal Science, Washington, D.C., October, 1967

2. Invited to present paper at Rochester Trophoblast Conference, Rochester, New York, October, 1967.

Publications:

1. Hopper, B.R., and Tullner, W. W.: Urinary estrogen excretion after ACTH in ovariectomized Monkeys (Macaca mulatta) Endocrinology 82: 876-877, 1968.

2. Tullner, W. W.: Urinary Chorionic gonadatropin excretion in the monkey (Macaca mulatta) early phase. Endocrinology 82: 874-875, 1968.

3. Kohler, P.O., Phang, J.M., Tullner, W.W., Ross, G.T., and Odell, W.W.: Effect of iodination on the biological activity and metabolism of human luteinizing hormone. J. Clin. Endoc. & Metab. (1968, in press)

4. Tullner, W.W.: Chorionic gonadotropin in the pregnant rhesus monkey. Proceedings of the Fourth Rochester Trophoblast Conference (1968, in press)

5. Lewis, J.L., Brown, W.E., Hertz, R., Davis, R.C., Johnson, R.A.: Heterotransplantation of Human Choriocarcinoma in Monkeys. Cancer (May, 1968 in press).

6. Tullner, W.W. and Gray, Clinton, W.: Chorionic gonadotropin excretion during pregnancy in a gorilla. Proc. Soc. Exp. Biol. and Med. July 1968 (in press)

Serial No. HD-RP2/3
1. Reproduction Research Branch
2. Section on Endocrinology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Endocrine Factors in Reproduction

Previous Serial No.: HD-RP2, HD-RP3

Principal Investigators: Dr. Roy Hertz, Dr. William Tullner, Dr. John Wilson

Other Investigators: Mr. Wilbert Nixon, Dr. Wayne Bardin, Dr. Carl Hansen, and Mr. Richard Pierson.

Cooperating Units: Endocrinology Branch, NCI; Laboratory Aids Branch, DRS.

Man Years:

Total: 8
Professional: 2
Other: 6

Project Description:

Objectives:

1. To gain basic information on endocrine factors regulating reproductive processes in laboratory animals and to determine the effects of hormonal and non-hormonal compounds on specific stages of reproduction (ovulation, implantation)

2. To determine the effects of age, sex and pregnancy and mammotropic tumor hormones on hepatic microsomal enzyme metabolism in the rat.

3. To study subcellular compartmentalization of biotin and the effects of metabolic inhibitors on biotin subcellular distribution in rat liver.

4. To ascertain the anatomical and physiological basis for infertility in laboratory breeding stock and to investigate the loss of fertility with age.

5. To develop a screen for evaluation of the zygototoxic effect of various steroids and related compounds.

6. To elucidate the mechanism involved in the steroid-hormone induced sexual behavior patterns of sexual receptivity, nesting, sex attractants and related behavioral phenomena.

Methods Employed:

1. Normal animals on standard test diets under controlled conditions of lighting and temperature were used.

2. Standard methods were used for microbiological and isotopic determinations of biotin.

3. Standard methods for determination of hepatic microsomal enzyme activity were employed.

4. Animals with timed pregnancies are treated at varied points during tubal transport, implantation, and fetal development for effects on zygote or fetal survival and subsequent fertility.

5. The reproductive behavior patterns of grooming, copulation, nesting and care of the young are studied in several species in relation to the normal cycle and its replacement by hormones in the ovariectomized, adrenalectomized, or hypophysectomized animal.

Major Findings:

A. Study of the reproductive function of retired breeding female rabbits, two to three years of age, reveals that mating continues for many months after fertility drops nearly to zero. In young breeding control rabbits ovulation occurs after mating in about 98% of the animals, whereas in the senile animal ovulation is seen in only 60% under identical conditions. The ovaries of the non-ovulating senile females are grossly and histologically similar to those of the younger females except for some evidence of a larger mass of interstitial tissue. In the ovulating senile rabbit viable corpora lutea are formed and fertilization, tubal transport and implantation proceed normally except in those instances in which pyometrium and/or pyosalpingitis are encountered. There then follows very high incidence of fetal resorption beginning about mid-pregnancy and as term approaches practically no young are delivered. Among ten such animals one viable young was delivered whereas over 100 implantation sites were observed among 10 such animals autopsied at mid-pregnancy. Among the resorbing fetuses occasional gross malformations are observed.

These findings indicate a progressive loss of reproductive and possibly of genetic potential in the ova of the aging rabbit as well as some impairment in the function of the copulation induced pituitary-gonadal function related to ovulation. The question of the pertinence of these findings to the occurence of increasing congenital problems in babies born to older women is raised. Further cytogenetic analysis of the ova and young of these animals is indicated.

B. Mice with timed pregnancies are given test compounds at various stages following fertilization to determine their effect on the viability and quality of the young. A number of estrogenic steroids are found to be zygototoxic but no evidence of dissociation from hormonal activity is observed to date. Initial phases of a study of the relationship of zygototoxcity to oncolytic potency of a number of highly active oncolytic agents reveals little relationship between these two functions although marked damage to unfertilized oocytes as well as to the fetus during organogenesis has been reported by others. This discrepancy may rest on quantitative and temporal factors of drug administration and may not prove to be of true biological significance.

C. The copulatory reflex in the guinea pig is readily observed under laboratory conditions. Normally this specific behavior pattern is limited to the few hours of the ovulatory period during the 16 day estrous cycle. In the ovariectomized guinea pig this reflex response is readily induced by estrogen followed by progesterone. Initial studies indicate that a number of psychotropic drugs alter this steroid-induced behavior pattern without general central nervous system depression. Since these compounds are observed to have marked hypothalamic effects in man, this experimental system may provide a valuable model for the study of the pharmacological alteration of steroid-dependent behavior patterns.

D. Gross anatomical dissection of infertile couples of line 2 inbred guinea pigs reveals no obvious basis for the relatively high frequency of infertility in this strain. The genital tract, adrenals, and thyroid of both the male and female partner are found to be grossly normal and abundant viable sperm of normal morphology are found in the electro-ejaculate as well as in the epididymis of the male partner. The ejaculate is also normal in volume and consistency and coagulates normally. The infertility of these couples merits further endocrine and immunological study.

E. The effects of pituitary mammotropic (MtT) tumor on hepatic drug metabolism in rats has been investigated further. This tumor secretes large quantities of somatotropin, prolactin and adrenocorticotropin. A decrease in the metabolism of 8 drugs by liver from MtT tumor bearing rats was observed after 30 days of tumor growth. Detailed observations were made on hepatic metabolism of hexobarbital and the formation of formaldehyde from aminopyrine. The decrease in liver drug metabolism not seen: (1) in rats bearing a transplantable adenocarcinoma (R3230AC); (2) when tumor homogenate, serum or liver from tumor bearing rats was added to the <u>in vitro</u> reaction mixture. MtT tumor effects on liver drug metabolism were not altered by adrenalectomy or orchiectomy. Removal of the MtT tumor was followed by a return of liver drug metabolism to normal levels.

The administration of a combination of the three pituitary hormones (STH, ACTH, and prolactin) produced by the MtT tumor decreased liver metabolism of hexobarbital and the production of formaldehyde from aminopyrine as compared with controls treated with albumin-saline or a similar STH, ACTH and prolactin which had been boiled. These findings suggest that the decreased hepatic microsomal metabolism found in rats bearing the MtT tumor may be due to the

pituitary hormones and/or other unknown tumor secretory products.

F. Biotin-deficient rats exhibit the following compartmentalization of biotin when compared with the pair-fed supplemented controls: (a) nuclear fractions contain a smaller percentage of total biotin (b) supernatant fractions contain a significantly larger percentage of total biotin. Metabolic inhibitors did not consistently change the amount of total biotin associated with a particular subcellular fraction. ^{3}H-biotin in the liver was found to have a biological half-life of about 50 days.

G. Continued observations on the effects of the pituitary mammotropic tumor on the adrenal-gonadal system show that: (1) testicular function is depressed in the male tumor-bearing rat; (2) adrenalectomy reverses this antiogonadal action: (3) that in orchiectomized tumor-bearing rats, androgenic stimulation of the ventral prostate gland occurred, and (4) the androgen has been identified as testosterone which is secreted by the adrenal in response to the tumor hormones (adrenocorticotropin, somatotropin and prolactin).

Proposed Course of the Project:

1. Investigation of the initiation, development and inhibition of the decidual reaction in rodents is continuing.

2. Studies on hepatic drug metabolizing enzyme systems in tumor-bearing rats will be discontinued when the principal investigator leaves NIH in June 1968.

3. The studies on infertility in breeding stocks and in senile rabbits will be extended substantially.

4. The search for acceptable zygototoxic agents are to be extended in both mice and in rabbits with a view to ultimate application to sub-human primates and man.

Significance to Bio-Medical Research and the Program of the Institute.

Further knowledge of the processes regulating reproduction may be expected to provide improved methods of sterility and fertility control as they have in the past.

Honors and Awards: Dr. Roy Hertz

1. Appointed Professor of Obstetrics and Gynecology (Human Development): The George Washington University Medical School, Washington, D.C.

2. Invited to address the Sixth World Congress on Fertility and Sterility, Tel-Aviv, May 19, 1968.

3. Invited to address the Nordwest deütsche Gesellschaft für Gynäkologie, Hamburg, Germany, May 10, 1968, and to address the School of Medicine at the Universitäts Krankenhaus Eppendorf, Hamburg, Germany.

4. Presented lecture in Cancer Chomatherapy Course, University of North Carolina, School of Medicine, Chapel Hill, North Carolina, April 20, 1968.

5. Appointed Chairman, Subcommittee on Carcinogenesis, Food and Drug Advisory Committee on Obstetrics and Gynecology.

6. Member, Program Committee, Tenth International Cancer Congress, Houston, Texas - 1970

7. Chaired a session on Clinical Chemotherapy, American Association for Cancer Research, Atlantic City, April 13, 1968.

8. Appointed to Editorial Board, Journal of the Society for the Study of Reproduction.

9. Member of World Health Organization, Task Force on Oral Contraceptives, October 1967, Geneva, Switzerland.

10. Chairman, Fourth Rochester Trophoblast Conference, University of Rochester, October 30, 1967.

Honors and Awards: Dr. William W. Tullner

1. Lecturer in Endocrine Chemistry, Georgetown University Graduate School, Washington, D. C., 1960 to present.

2. Consultant to Graduate Council (Endocrinology and Metabolism), The George Washington University, Washington, D.C. 1967 to present.

Honors and Awards: Dr. John T. Wilson

1. Invited to participate in seminar on "Pituitary Hormones and Drug Metabolism," University of Iowa, Department of Pediatrics, December 1967.

2. Invited to present paper on "Liver drug metabolism in rats bearing a pituitary tumor" and seminar, Department of Pharmacology, State University of New York, Buffalo, New York, March 1968.

3. Participated in seminar on "Neonatal Pharmacology," Department of Pediatrics, Columbia College of Physicians and Surgeons, New York, New York, March 1968.

Publications:

1. Wilson, John T.: An investigation of the decrease in the hepatic metabolism of hexobarbital, aminopyrine and p-nitrobenzoic acid in rats bearing a pituitary mammotropic tumor. J. Pharm. Exper. Ther. 160: 179-188, 1968

2. Wilson, John T.: The effect of a pituitary mammotropic tumor on hepatic microsomal drug metabolism in the rat. Biochem Pharm. 17: (in press) 1968.

3. Wilson, J.T. and Fouts, J.R.: Hepatic microsomal drug metabolizing enzyme activity in the opossum. Biochem. Pharm. 16: 215-218, 1967.

4. Gram, T.E., Wilson, J.T. and Fouts, J.R.: Some characteristics of hepatic microsomal systems which metabolize aminopyrine in the rat and rabbit J. Pharm. Exper. Therap. 158: 172, 181, 1967.

Serial No. HD-RP 10 (c)
1. Reproduction Research Branch

3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Human Reproduction

Previous Serial No. None

Principal Investigators: Dr. Roy Hertz, Dr. William W. Tullner and Mr. Wilbert Nixon

Other Investigators: Dr. Griff Ross, Dr. M.B. Lipsett, Dr. W.D. Odell, Dr. J.T. Sites (George Washington University Hospital) Dr. C.B. Hammond, Dr. J.A. Lewis, and Dr. Willis Brown, Jr.

Cooperating Units: Endocrinology Branch and Surgery Branch, National Cancer Institute, Department of Obstetrics and Gynecology, The George Washington University Medical School.

Man Years:

Total: 6
Professional: 2
Other: 4

Project Description:

Objectives:

To elucidate problems related to human reproduction with special emphasis on: (a) fertility control and family planning, (b) sterility (c) gonadal development and pubescence, (d) menopausal and post-menopausal phenomena, (d) disorders of menstrual function, (f) normal and abnormal pregnancies including such problems as habitual abortion, missed abortion, therapeutic and criminal abortion, ectopic pregnancy, and placental abnormalities such as placenta previa, abruptio placenta, hydatidiform mole, and choriocarcinoma.

Methods employed:

1. Patients are observed in both a hospital and out-patient setting for complete diagnostic study and for therapeutic management under conditions of detailed clinical control and in accordance with previously developed research protocols. Comprehensive record-keeping and follow-up are

maintained.

2. Epidemiologic observations are made in a community setting either by direct observations or by analysis of data already set forth in clinical records.

3. Correlated biochemical, morphological and biological studies are carried out on tissues and body fluids of patients in various phases of treatment or observation in relation to the clinical problems listed under "objectives" above. Most frequent methods used are: bioassay and immunoassay for protein hormones, steroid hormone analysis by gas chromatography, histochemical and morphological analysis of vaginal smears and endometrial, ovarian, and cervical biopsies.

4. Studies of the effect of various endocrinological alterations on fat, carbohydrate, protein and mineral metabolism are conducted.

Major Findings:

1. A comprehensive review was made of the unresolved problems connected with the use of the oral contraceptive steroids with major emphasis on the problem of the carcinogenic potential of these preparations. In addition, the problem of the long-term effect of the steroid contraceptives on the genetic potential of the remaining ova in the human ovary was surveyed. These observations have led to the formulation of long-term epidemiological studies for the resolution of these questions. (Publications 1 and 2)

2. A detailed review of existing knowledge concerning the carcinogenic potential of various intra-uterine contraceptive devices was made and has provided the basis for recommendations for studies in this area to be developed on contract basis by the Food and Drug Administration. (Publication 3).

3. Continued studies of the nature and treatment of choriocarcinoma and related trophoblastic tumors in women have provided a full characterization of the endocrinological phenomena associated with this disease spectrum in women. (Publication 4). Clinical and laboratory studies establish the production of thyreotropin as well as gonadotropin by trophoblastic tumor of gestational orgin. In addition, the development of refractoriness of the patient's ovary to endogenous chorionic gonadotropin concomitant with continued responsiveness to endogenous pituitary gonadotropin is described (Publication 4).

4. Several medical diagnostic problems arising during the clinical course of choriocarcinoma and related trophoblastic neoplasms are fully analysed for the guidance of clinicians undertaking therapy in this area. (Publication 5).

5. A comprehensive survey of twelve years of experience in the study of gestational trophoblastic disease in women is in progress through the aid of a computerized analysis of our clinical, laboratory, and histopathological data. Portions of these data have been formally presented before numerous clinical groups. These data constitute the essential basis for the development of a regional program of laboratory and clinical service to practicing physicians who are confronted with patients with trophoblastic neoplasms.

6. A complete endocrinological and clinical characterization of sixteen women with persistent amenorrhoea and infertility following discontinuation of oral contraceptive use has been completed. The nosological findings suggest that young women, 18 to 22 years of age, are especially susceptible to this unfortunate development. All but two have recovered menstrual function and fertility after intervals varying from 3 to 18 months without any specific therapy. All laboratory parameters of adrenal, thyroid, and pituitary gonadotropic function were within normal limits. Profound endometrial atrophy as demonstrated by biopsy appears to be the common deficiency. Direct measurements of ovarian function were not carried out but no distinct pattern of primary gonadal failure was evident in the vaginal smear patterns.

Proposed Course of Project:

Pending the development of clinical resources of these studies continued cooperation with other groups at National Institutes of Health, U.S. Naval Medical Center and elsewhere is proposed for the continuation and extension of these studies. Access to family planning clinics and to an active gynecological and obstetrical service is essential and it is hoped that such resources may in time be developed within the National Institute of Child Health and Human Development's coordinated clinical activities on pre-natal, peri-natal,and developmental problems.

Honors and Awards: Dr. Hertz

1. Appointed Professor of Obstetrics and Gynecology (Human Development): The George Washington University Medical School, Washington, D.C.

2. Invited to address the Sixth World Congress on Fertility and Sterility, Tel-Aviv, May 19, 1968

3. Invited to address the Nordwest deütsche Gesellschaft für Gynäkologie, Hamburg, Germany, May 10, 1968, and to address the School of Medicine at the Universitäts Krankenhaus Eppendorf, Hamburg, Germany

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9. Member of World Health Organization, Task Force on Oral Contraceptives, October 1967, Geneva, Switzerland.

10. Chairman, Fourth Rochester Trophoblast Conference, University of Rochester, October 30, 1967

Publications:

1. Hertz, R., and Bailar, C.: Estrogen-Progestogen Combination for Contraception. Hormonal Steroids (L.Martini, F. Fraschini, M. Motta, eds) 841-851, 1967, Amsterdam.

2. Hertz, R.: Role of Steroid Hormones in the Etiology and Pathogenesis of Cancer. Am. J. Obst. and Gynec. 98, 1013-1019, 1967

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NICHD Annual Report
July 1, 1967 through June 30, 1968
Behavioral Biology Branch

This Branch is still in its very early stages of formation. At the beginning of FY 1968, Dr. Loring Chapman, who had been Chief of the Branch, left NIH to accept the Chairmanship of a University Department. Since that time, the Branch has been without a Chief, and the four professionals currently working in it have reported directly to the Scientific Director of the Institute. They have collaborated with the Children's Diagnostic and Study Branch in the electrophysiological and behavioral evaluation of children with disorders associated with mental retardation. Not only have they supplied routine EEG evaluation of the mentally retarded subjects being studied by the Children's Diagnostic and Study Branch, but they have also used this population of subjects for the development and improvement of tests utilizing evoked responses. Another, and a very fruitful, collaboration involves three of the professional members of this Branch. Using pregnant sheep, they have gathered a considerable volume of data relating neurophysiological (EEG) cardiovascular and metabolic observations on the fetus under hypoxia and during recovery from hypoxia. They seek to identify ways in which the fetal environment may be modified to improve resistance to hypoxia, shorten recovery from hypoxia, and increase survival following hypoxia.

A child psychologist within the Branch is initiating studies which will explore the developmental course of differential responses of infants to face stimuli during the first year of postnatal life.

While the professionals in this Branch are productively employed, the expansion of the Branch and the development of its program awaits the appointment of a Branch Chief.

Serial No. HD-BB1
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Acute fetal hypoxia - neurophysiological, cardiovascular and metabolic observations

Previous Serial Number: New Project

Principal Investigator: Leon I. Mann

Other Investigators: James W. Prichard, David Symmes

Cooperating Units: Laboratory Aids Branch, Division of Research Services

Man Years:

Total:	3.0
Professional:	1.75
Others:	1.25

Project Description:

Objectives: The objectives of the project are (1) to obtain basic neurophysiological, cardiovascular, and metabolic observations from the fetus under hypoxia (2) inter-relate these parameters temporally and functionally in terms of impairment, recovery and survival (3) modify the alterations to improve resistance to hypoxia, shorten recovery and increase survival.

Methods Employed: Thirty-six gravid ewes have been studied. Hypoxia at levels of 15%, 7.5%, 5% and 0% is induced through a maternal tracheostomy tube utilizing anesthetic apparatus. Catheters are placed so that simultaneous samples can be obtained from the maternal artery, uterine vein, umbilical artery, umbilical vein and amniotic fluid. Maternal and fetal EEG and EKG electrodes are placed, and constant recording is obtained. Maternal and fetal blood pressure is monitored. Blood volume studies are performed. Catheter samples are drawn prior to, during and following hypoxia at predetermined intervals and analyzed for pH, pCO_2, pO_2, glucose, lactate, pyruvate, hemoglobin and hematocrit. If experimental conditions permit a second hypoxic period is induced.

In approximately 10 animals a glucose load was given the mother prior to hypoxia. Time samples were obtained following infusion and throughout the experiment.

At no point is the fetus or umbilical cord delivered outside the uterine cavity.

Major Findings: Data analysis is presently in progress: Three major findings seem forthcoming.

1) A clear description of the temporal relationship of the EEG, cardiovascular and metabolic alterations during and following hypoxia with or without glucose administration. Functional interaction appear to be present among these varables.

2) An ellucidation of the acid base changes occurring during hypoxia with clear evidence for a metabolic (lactate, base excess) rather than a respiratory (pCO_2) problem. Placental regulation of fetal lactate and pyruvate is probable, and the data are under further analysis.

3) An understanding and ellucidation of glucose transport and utilization by the fetus.

Significance to Biomedical Research and the Program of the Institute: Simultaneous observations of neurophysiological cardiovascular and metabolic parameters during and following acute hypoxia with the fetus in utero are unavailable in the literature. The data obtained from this study provide this information. Hopefully additional research may be directed toward preventing or correcting the alterations observed.

Because the human fetus is unaccessible to studies of this sort, animal experimentation is invaluable in fetal physiology. Knowledge and understanding of human development and mental retardation, two goals of the Institute, are clearly dependent upon a clear concept of normal and abnormal conditions existing in lower animals. This project is intended to provide information of this sort.

Proposed Course:

1) To obtain blood flow data on both the uterine arterial and fetal systems while simultaneously measuring oxygen content. The former will be obtained with use of an ultrasonic flow meter, the latter measured by the Van Slyke Manometric apparatus. The product of flow times arterial-venous difference of oxygen will allow calculation of the oxygen consumption prior to, during and following hypoxia for the total uterine-placental-fetal complex as well as the fetal complex independently. From this data calaculations of the placenta diffusion capacity is possible furthering our understanding of the maternal-fetal regulation of oxygen.

2) Efforts will be made to correct the fetal metabolic acidosis, so clearly demonstrated by the data, by exogenously administering various substrates and alkalinizing agents to the mother.

3) It is anticipated that the preparation will allow adequate study of the drug effects on the fetal central nervous system.

4) An attempt will be made to obtain this information as well as other research stimulated by an analysis of the present data from both acute and chronic preparations.

Honors and Awards: None

Publications: None

Serial No. HD-BB2
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Propagation of abnormal discharges in the leech nervous system

Previous Serial Number: None

Principal Investigator: James W. Prichard

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Others:	0.0

Project Description:

Objectives: Facilities are being developed for studying the propagation through the leech nervous system of abnormal discharges induced by metrazol, penicillin, tungstic acid, freezing and other means.

Methods Employed: Microelectrode recording of electrophysiological endpoints will be made before and during treatment with the agents and conditions mentioned above.

Major Findings: None. The project is still in its early phases.

Significance to Biomedical Research and the Program of the Institute: The leech nervous system has considerably fewer neurons and a much simpler architecture than the vertebrate nervous system. These characteristics lend themselves to a more exhaustive analysis of the spread of excitation in the presence of the drugs employed than might otherwise be possible.

Proposed Course: Thus far, time has been given exclusively to assembling and testing equipment. Between now and 30 June 1969, the proposed studies will be carried out.

Honors and Awards: None

Publications: None

Serial No. HD-BB3(c)
1. Behavioral Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Behavioral and psychophysiological studies of mentally retarded children

Previous Serial Number: None

Principal Investigator: David Symmes (initiated by Loring Chapman)

Other Investigators: James W. Prichard

Cooperating Units: Children's Diagnostic and Study Unit, NICHD

Man Years:

Total:	11/12
Professional:	8/12
Others:	3/12

Project Description:

Objectives: This project seeks to improve methods for behavioral and psychophysiological assessment of children with disorders associated with mental retardation.

Methods Employed: Telemetered and direct-wire recording of evoked responses from children.

Major Findings: Analysis of evoked responses stored on instrumentation tape indicate a suboptimal frequency fidelity. Twenty retarded and a smaller number of normal subjects have thus far been recorded.

Significance to Biomedical Research and the Program of the Institute: Improved technical capabilities to accurately record evoked potentials would help to extend our knowledge of normal electrophysiological responses to specific stimuli and stimulus patterns. Improved techniques would also make it possible to determine the value of evoked potential studies for distinguishing and characterizing the several types of central nervous deficiency now called mental retardation.

Proposed Course: To add additional subjects as they become available. To improve the stimulus programming. To improve the fidelity of the data acquisition steps.

Honors and Awards: None Publications: None

Serial No. HD-BB4(c)
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Infant attention: visual fixation and heart rate responses of the 3 to 9 month old human subject

Previous Serial Number: None

Principal Investigator: James M. Weiffenbach

Other Investigators: None

Cooperating Units: Department of Pediatrics, National Naval Medical Center, Bethesda, Maryland

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: This project seeks to explore the developmental course of differential responses to face stimuli as exhibited by human infants during the first year of life.

Data on the issue of infant's discrimination of his mother from a stranger will come from a comparison of each infant's response to a projected photograph of his own mother's face with his responses to a similar photograph of the mother of another infant from the same sample (stranger). Such basic data are needed for a more solid understanding of infant social development, e.g. stranger anxiety.

In pursuit of a second major objective, attentional responding to distortions of face stimuli will be explored to discover the relation of this effect to prior long term experience with the specific face that undergoes distortion. Such data are crucial for the discrepancy hypothesis which maintains that the attentional responding to distortions are based on a discrepancy between current stimulation and centrally stored effects of previous experience.

Methods Employed: Independent samples of infants will be tested at ages 3,4, 5,6,7, and 8 months. Each infant will be re-tested one month after his initial session. Visual fixation and heart rate response will be recorded as each infant views the photographic projection of the face of his mother and that of another mother in the sample. Both the mother and stranger will be displayed

in undistorted and distorted form. This design is unique in that it provides for manipulation of the relation of the infant to the person represented in the distorted face stimuli. In addition this project differs from previous work in that age range coverage has been improved and the bias associated with using a single "stranger" has been avoided.

Major Findings: This program is just beginning and thus no findings are available at this time.

Significance to Biomedical Research and the Program of the Institute: The research to be carried out under this project will have significance for biomedical research and contribute to the program of the Institute in as far as it provides data relevant to an understanding of the normal course of perceptual and cognitive development during the first year of life. During the past decade, both the scientific and the lay community have attached increasing importance to early development as a determiner of later intellectual function. However, the burden carried by speculation concerning cognitive and intellectual mechanisms as they develop during the first year has not been balanced by adequate systematic investigation of infant behavior. It is hoped that this project will contribute to the redress of this unbalance.

Proposed Course of Project: This new project will begin testing subjects before the close of this fiscal year and be completed during the next fiscal year.

Honors and Awards: None

Publications: None

NICHD Annual Report
July 1, 1967 through June 30, 1968
Social and Behavioral Sciences Branch

Summary of Research Activities

The research program of the Social and Behavioral Sciences Branch is directed towards understanding the processes underlying developmental changes in perceptual, cognitive and personal-social functions; the interrelationships among these functions, and the interactions between biological and environmental determinants of behavior.

Current research is focused on the infancy and preschool periods with a concentration on children from "disadvantaged environments." These studies are aimed primarily at increasing our understanding of basic developmental processes. By studying extreme environmental conditions, it is hoped to delineate more sharply some of the variables significant for normal development. In addition this research will add to specific knowledge of the characteristics and environmental conditions of children from lower class social backgrounds. Until recently research data on the developmental characteristics and the psychological, as distinguished from the sociological, characteristics of lower social class environments has been meager. More differentiated knowledge is needed to provide solid foundations for social action programs aimed at preventing and ameliorating the disabilities commonly found in children from these backgrounds.

The program of research on early influences on cognitive and personal-social development consists of two major projects which include several interrelated studies. In the research on the environmental correlated of development during the first six months of life, a major share of data collection has been completed. Data on environmental stimulation and patterns of early infant care have been obtained on 55 subjects from lower to lower middle class backgrounds. In these families, infants have been studied with regard to cognitive characteristics, exploratory behavior, motor development, and social responsiveness. Comparable data are now being obtained on a group of 15 subjects extending the range into the middle class.

In the investigation of preschool learning environments and the intellectual and personality characteristics of preschool children from culturally deprived backgrounds, some methodological studies have been conducted, and a substudy on cognitive-personality interrelationships has been completed.

A major concentration of effort during the past year has been on the development and pre-testing of methods for analyzing the components of preschool learning environments. An observational system has been developed for studying various dimensions of teacher behavior, peer influence and child behavior in the classroom. Observations have been made in several types of preschool settings with different educational philosophies, e.g., Montessori, relationship-centered. Techniques have been developed and are being tested for assessing some aspects of behavioral controls, such as, capacity to delay gratification.

Analyses of cross-sectional data on a sub-sample of 20 cases give some support to the basic hypothesis of a significant relationship between the development of behavioral controls and verbal expressive behavior. Significant positive relationships were found between measures of behavioral control and indices of capacity for verbal elaboration.

In the research on the development of stranger anxiety during infancy being carried on in collaboration with the Child Research Branch of National Institute of Mental Health, data collection has been completed. A report on one facet of the study - the relationship of paternal behavior to the development of social attachment was present at the annual meeting this year of the American Orthopsychiatric Association. Further data analyses are in progress on the significance for the development of attachment of a number of variables, such as, maternal social stimulation; amount of eye-to-eye contact.

During the past year a conference was held on methodological issues in research on mother-infant interaction. A number of investigators actively involved in studies of early mother-infant relationships discussed their current work, with emphasis on concepts and methodology. Part of the discussion focused on clarification of the concept of attachment and the meaning of the various behavioral indices that have been used to define attachment. Among the methodological issues considered were the relations between choice of observational categories and basic theoretical orientations, approaches to contingency analyses in observational studies, and basic problems of observer reliability.

Serial No. HD-GD3

1. Social and Behavioral Sciences Branch
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies of Intellectual and Personality Development of Pre-School Children from Culturally Deprived Backgrounds

Previous Serial Number: Same

Principal Investigators: Leon J. Yarrow, Ph.D.; Michael Fishman, M.D.;[1] Frank Pedersen, Ph.D.

Other Investigators: Joseph J. Jankowski, M.D.; Joan Gildemiester, M.A.: Bette Marcus, M.A.

Cooperating Units: Office of Economic Opportunity, Project Headstart

Man Years

Total:	3.0
Professional:	1.5
Others:	1.5

Project Description:

Objectives: This investigation has focused on two major problems: the relationships between cognitive characteristics and behavioral controls in preschool children; and the relationship between preschool learning conditions and cognitive and personal-social development of preschool children.

Methods Employed: Time-sampling observational methods are being used to study several aspects of the preschool environment: teacher control, cognitive stimulation, affective interaction with the teacher, and peer influences. For studying the dependent variables, standard tests of intellectual development are being used, and measures of specific cognitive and language functions are being adapted. The assessment of behavioral controls will be made through structured situations as well as through time-sampling observations of child behavior in the classroom.

Major Findings: Data on cognitive characteristics and on several dimensions of behavioral controls were obtained on a sample of children in Headstart

[1]Staff member until December 30, 1967

schools in the District of Columbia. Analyses of these cross-sectional data give some support to the basic hypothesis of a significant relationship between behavioral controls and language development.

A major concentration of effort during the past year has been on refining concepts and the development of techniques for studying preschool learning environments. An observational system has been developed and pretested for studying a number of aspects of the preschool setting: teacher control, cognitive stimulation, affective interaction; and peer influences. In addition, work has continued on developing methods for assessing behavioral controls by direct observation of child behavior in the classroom, and through structured situations for assessing such aspects of behavioral control as frustration tolerance, motor inhibition, capacity to delay gratification, and to sustain focused attention.

Significance to Bio-medical Research and the Program of the Institute: Although there has been much research on basic learning processes in restricted experimental situations, the findings and concepts have had limited generalizability to more complex learning settings. Current controversy about the relative merits of various approaches to preschool programs emphasizes the need for greater conceptual clarity about the preschool environment. The concepts and techniques developed in this research should contribute to more adequate methodologies for studying complex environmental inputs in relation to the cognitive and personal-social development of preschool children. The substantive findings should add to understanding of the factors which influence cognitive development and the development of behavioral controls during the preschool years. These data should also have direct implications for developing preschool and day care programs for young children.

Proposed Course of Project: It is anticipated that the methodological phase of the study will be completed in the early part of the next fiscal year. After completion of pre-testing of the observational techniques and the situational measures for behavioral controls, data collection will be initiated in several Headstart schools in the greater Washington area.

A conference has been planned this year to consider approaches to conceptualizing and measuring preschool learning environments. The major focus will be on articulating concepts from several theoretical orientations relevant to preschool education, e.g., operant learning, social learning, psychodynamic-holistic approach and cognitive learning.

Honors and Awards: None

Publications: None

Serial No. HD-GD4
1. Social and Behavioral Sciences Branch
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Early Environmental Influences on Cognitive and Personality Development in Infancy

Previous Serial Number: Same

Principal Investigators: Leon J. Yarrow, Ph.D., Judith Rubenstein, Ph.D., Frank A. Pedersen, Ph.D., Joseph J. Jankowski, M.D.

Other Investigators: Myrna Fivel, M.A.

Cooperating Units: D.C. Department of Public Health, Children's Hospital of the District of Columbia, Group Health Association of Washington, D. C.

Man Years

Total:	5.25
Professional:	2.25
Other:	3.

Project Description:

Objectives: This is the first in a series of investigations of early environmental influences on cognitive and personality development during the first year of life. In this project the major goals have been to identify the early antecedents of the cognitive deficits and disturbances in personal-social functioning which are commonly found in children from low socio-economic backgrounds.

Methods Employed: The main sample consists of 55 infants and their caretakers from low socio-economic backgrounds obtained through two well-baby clinics in the District of Columbia. To extend the range of environmental variables and to permit some exploration of the effects of social class status on early maternal care, a sample of 15 infants from a middle-class background has been obtained through a prepaid comprehensive medical care program. Comprehensive pediatric and neurological examinations were conducted to exclude infants with physiological abnormalities that might be prognostic of developmental retardation.

Data on environmental variables have been obtained at five months through time-sampling observations in the home using pre-coded categories.

Data on infant characteristics have been obtained by means of developmental examinations, tests of selected cognitive functions, a measure of exploratory behavior and measures of social responsiveness.

Major Findings: Data collection has proceeded on schedule. Data on environmental stimulation and patterns of maternal care, and on infant cognitive and social characteristics have been obtained on 55 subjects from lower social class backgrounds. Comparable data are now being gathered on a sample of 15 middle class families.

The initial steps on the research consisted of development and refinement of research instruments. A comprehensive system of codes was developed for direct observation in the home to tap a number of aspects of maternal stimulation, e.g., eye-to-eye contact with the infant, contingent vocalization, social play, smiling, cuddling, ways of responding to the infant's cry. Rating scales were developed to summarize more global aspects of the home environment and aspects of maternal behavior not appropriate for time-sampling observations. In addition, a coding scheme for analyzing stimulus properties of toys and household objects was developed, e.g., the extent to which each play object within reach of the infant is responsive to the young baby's exploratory manipulation in terms of potential visual, tactile, and auditory feedback, e.g., moving parts, pliability of materials, and the kinds of sounds produced by manipulation.

The first steps of data analysis are underway: coding social background data, summarizing test and observational data. Detailed planning for computer analyses are in progress.

Significance to Bio-medical Research and the Program of the Institute: Data on the relationship between parameters of stimulation in the home and developmental progress during infancy will give more precise understanding of the environmental factors which facilitate and those which hinder intellectual and personal-social development. Such findings should have direct relevance to current social programs for underprivileged families.

The techniques developed have been found useful for analyzing complex environments in the first year of life. These methods can, with some adaptations, be used to study environmental influences in many different types of settings, e.g., day care and residential care programs for young infants.

Proposed Course of Project: Data collection will be completed by July 1968. The major activity during Fiscal Year 1969 will be concerned with analysis and interpretation of data and the preparation of reports on the findings.

Honors and Awards: None

Related Publications:

1. Pedersen, Frank A., and Wender, Paul H.: Early Social Correlates of Cognitive Functioning in Six-Year-Old Boys. Child Development, 39: 185-193, March 1968.

2. Rubenstein, Judith: Maternal Attentiveness and Subsequent Exploratory Behavior in the Infant. Child Development, 38: 1089-1100, December 1967.

3. Yarrow, Leon J. The Development of Focused Relationships During Infancy. In Hellmuth, Jerome (Ed.): Exceptional Infant: The Normal Infant, Vol. I., Seattle, Wash., Special Child Publications, 1967, 429-442.

Serial No. HD-GD5
1. Social and Behavioral Sciences Branch
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Antecedent and Concurrent Correlates of Infantile Stranger Anxiety

Previous Serial Number: Same

Principal Investigator: Frank A. Pedersen, Ph.D.

Other Investigators: Kenneth S. Robson, M.D.
Tufts New England Medical Center
Boston, Massachusetts

Howard A. Moss, Ph.D.
Child Research Branch
National Institute of Mental Health

Cooperating Units: Child Research Branch, National Institute of Mental Health

Man Years

Total:	0.2
Professional:	0.1
Others:	0.1

Project Description

Objectives: This study has a principal focus on the development of social attachments and a related phenomenon "stranger anxiety" during the first year of life. It is concerned with the role of specific aspects of maternal behavior and mother-infant interaction in the development of these social behaviors. In addition, the range of paternal participation in infancy was studied and its significance for the development of attachment and stranger anxiety was examined.

Methods: The sample consists of 46 first born infants, half males and half females. Each infant was observed at 8 months and at 9½ months in the home with the mother present. Data were obtained through observations and ratings of infant's reactions to specific interventions by the experimenter, and through a structured interview with the mother.

Major Findings: Among other findings, stranger anxiety and spontaneous social approach behavior in infants are correlated with particular patterns of social stimulation observed at one month and at three months of age. For example, infants who received higher levels of stimulation through the distance receptors (auditory and visual stimulation) through the mother's talking or singing and positioning the baby so that it could see the mother's face were significantly lower in stranger anxiety at 8 months and at 9½ months. The frequency of eye-to-eye contact between mother and baby at one month of age was predictive for males of spontaneous social approach to the experimenters and the extent to which the infant looked directly at the experimenter's face when held. Although stranger anxiety has frequently been indexed only by crying or protest, the infant's visual behavior also appears to be a sensitive measure of his reaction to strangers.

Another set of findings points to the significance of a relatively neglected aspect of early experience--father-infant relationships. Several dimensions of paternal behavior are highly related to the age of onset and intensity of attachment to the father. For boys, the amount of caretaking activities in which the father engaged and the stimulation level of his play with the infant were positively correlated with the development of attachment to the father. The father's irritability level was negatively correlated with attachment. These results were presented at the annual meeting of the American Orthopsychiatric Association.

Significance to Bio-medical Research and the Program of the Institute: These findings provide more differentiated understanding of the role of specific patterns of maternal stimulation in early infancy in the development of social approach and avoidance behavior with strangers.

Proposed Course of Project: The immediate aims of the project will be to complete reports of the basic data which have been outlined in this report.

Honors and Awards: None

Publications: None

NICHD Annual Report
July 1, 1967 through June 30, 1968
Laboratory of Biomedical Sciences

The Laboratory of Biomedical Sciences investigates abnormal physical and mental development at several levels of organization. To further the objective of establishing a multidisciplinary environment which will foster this type of research three sections have been formed during the past year: Section on Developmental Enzymology, Section on Intermediary Metabolism, and Section on Physiological Controls. The Laboratory needs further strengthening in the areas of Molecular Structure, Developmental Genetics, and Molecular Biology. When this organizational structure has been attained, the Laboratory will be capable of examining many facets of specific problems in developmental pathology: aberrations in the genetic informational content of cells, whether arising by gene mutation or by more gross chromosomal abnormalities; variation in the expression of the genetic information as reflected in abnormal enzymes and other types of polymorphism; the developmental consequences of altered metabolism resulting from defective enzymes; the hormonal mechanism for the induction and control of enzyme systems in the developing organism; and, finally, the elucidation of the structural basis for variants of biologically important macromolecules.

Among the more significant research results obtained in the Laboratory during the past year are:

1. Cytogenetic examination of 172 abortuses revealed that triploid and XO aneuploidy are the most frequent chromosomal aberration encountered in spontaneous abortion specimens. Viruses and mycoplasma have been detected in some of the specimens, but these findings have not been completely evaluated.

2. The regional and temporal distribution of 4 enzymes (methionine-activating enzyme, cystathionine synthase, D-amino acid oxidase, and esterase) in the developing Rhesus monkey brain is being analyzed. This work indicates that cystathionine may be produced in areas rich in nerve cell bodies, and that transsulfuration is as active, and perhaps more so, in the early fetus as in the more mature animal. Isozymes of D-amino acid oxidase were detected in 7 of 19 areas of the juvenile monkey brain; a multiplicity of esterases was detected in all of the areas.

3. The fetal rise in liver cystathionine synthase activity was shown to be related to new enzyme synthesis by use of an inhibitor of protein synthesis. The rise could not be prematurely induced by any of a number of hormones.

4. The technique for the isolated perfusion of rat livers has been used to gain further insight into the mechanism of action of hormones at the molecular level. The findings that both cyclic AMP and 5'-AMP directly activate liver phosphorylase and inactivate UDPGlucose-glycogen transferase may provide an explanation for the dual control of glycogenolysis in liver by hormonal (cyclic AMP-mediated) and non-hormonal (5'-AMP-mediated) mechanisms as occur with hypoxia.

5. The mechanism whereby ACTH regulates the growth and metabolic function of adrenal tissue is being investigated. The results suggest that ACTH and cyclic AMP may regulate adrenal protein synthesis at the translation level and this may be of importance in the steroidogenic effects of these substances.

Serial No. _____
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Role of Viruses in Chromosome Abnormalities

Previous Serial Number: HD-MR12

Prinicpal Investigators: J. D. Singer
R. L. Kolodny

Other Investigators: J. L. Sever
W. London
D. Madden

Cooperating Units: J. L. Sever, Section on Infectious Diseases, Perinatal Research Branch, NINDB
K. Sell, Tissue Bank, National Naval Medical Center, Bethesda, Maryland
R. McCallum, Kaiser Permanente Hospital, Honolulu, Hawaii

Man Years

Total:	34/12
Professional:	13/12
Others:	21/12

Project Description:

Objectives: More and more information is being accumulated showing that viruses have rather profound effects on chromosome morphology. Such effects include gaps, breaks, pulverization, and rearrangements. For example, clinical measles results in chromosomal changes, as does viral infection of various tissue cultures. The aim of our laboratory is twofold: to correlate the cytogenetics of human abortion tissue with virological studies, and to further analyze chromosomes of patients with viral illnesses. Thus more information will be gathered on the interesting relationship between viruses and chromosomes.

Methods Employed: Routine tissue culture methods are being used to grow abortus tissue and to produce chromosomes for study. Viral and mycoplasma studies are made on the abortus specimens, and paired sera are obtained from both parents for titer levels.

Routine blood cultures are done where the association of clinical virus infection with chromosomes is being studied. Such cases include subacute sclerosing panencephalitis (SSPE) and rubella.

Major Findings: To date, 172 spontaneous abortion specimens have been received from the cooperating Hawaii group. Many of the studies are still in progress; however, several triploid and XO abortuses have been found. In addition, an interesting viral isolation is pending from several large fetuses received in a 2½ week period. Mycoplasma has also been isolated from approximately 20% of the specimens.

In the study of SSPE patients, several have been found to have chromosomal gaps, breaks, and pulverization. In the other virus infections studies, it is too early to present any clear-cut findings.

Significance to Biomedical Research and the Program of the Institute: This study is expected to add to the knowledge of the possible role of viral and mycoplasma infections in the occurrence of human abortion. In addition, further efforts are being made to more fully elucidate the relationship of viral infection to chromosomal abnormalities, via the abortion project and selected clinical material.

Proposed Course of Project: Investigations are continuing on the role of viruses and mycoplasma infection in relation to chromosomal abnormalities in spontaneous human abortion. This project will terminate within the next year.

Further studies are being made on the role of viral infection in chromosomal changes of the infected person. These studies may be extended to viral infection of tissue culture.

Honors and Awards: None

Publications: None

Serial No. HD-LB1

1. Laboratory of Biomedical Sciences
2. Section on Developmental Enzymology
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Developmental Enzymology

Previous Serial Numbers: HD-MR1, HD-MR4(7)(c), and HD-MR5(7)

Principal Investigator: J. C. Robinson

Other Investigators: J. Volpe

Cooperating Units: W. T. London, Institute for Cancer Research, Philadelphia, Pennsylvania
H. Marques-Monter, University of Mexico, Mexico, D. F.

Man Years

Total:	8/12
Professional:	2/12
Others:	6/12

Project Description:

Objectives: The main objectives of this project are: (1) to identify and characterize pregnancy-associated enzymes and "carrier" proteins in maternal blood plasma; (2) to isolate and characterize enzymes and other biologically active molecules from the placenta; and (3) to investigate selected enzyme systems in the developing embryo, fetus, and neonate.

Methods Employed: The principal methods employed are: (1) the zymogram technique, by which electrophoretic variants of enzymes can be detected; (2) autoradiography, with which potential carrier function is evaluated (for example, by using I^{131}-tagged thyroxine); (3) enzymatic assays, which are usually based on spectrophotometric methods; (4) protein isolation techniques, including salt- and solvent-fractionation, gel-filtration, and ion-exchange chromatography; and (5) immunochemical procedures.

Major Findings: (1) Isoenzymes of D-amino acid oxidase were detected in 7 of 19 regions of the juvenile Rhesus brain. (2) Esterase zymograms revealed 10 to 15 components in all 19 regions of the Rhesus brain. (3) Preliminary experiments demonstrated the usefulness, for developmental studies, of the general zymogram technique for peroxide-producing enzymes, which was developed in this laboratory. (4) Evidence was obtained which suggests a mutually exclusive relationship between the appearance of the normal

pregnancy-associated serum cystine aminopeptidase$_2$ and an aminopeptidase detected in the serum in molar pregnancies. (5) An improved method for detecting electrophoretic variants of alkaline phosphatase was developed.

Significance to Biomedical Research and the Program of the Institute: These investigations attempt to extend knowledge of normal and abnormal developmental processes beyond the levels of gross and microscopic observations to the molecular level. When the contribution of the placenta and other fetal tissues to the appearance of the pregnancy-associated enzymes in maternal serum has been evaluated, a firm basis will be established for a search for correlations which may exist between abnormal pregnancies and variations in the plasma components. The ease with which maternal blood samples can be obtained is one of the prime reasons for undertaking this approach to the detection of intrauterine developmental pathology.

Proposed Course of Project: (1) The regional and temporal distribution of D-amino acid oxidases, esterases, and other enzymes in the developing primate brain will be investigated. (2) Studies on selected placental enzymes will be undertaken. (3) Investigation of the pregnancy-associated maternal plasma enzymes will continue in order (a) to determine the source of these proteins, i.e., to establish whether they are derived from maternal or fetal tissues; (b) to study the genetic variation (polymorphisms, etc.) which exist among the proteins; (c) to establish the norms for the time of appearance and the levels attained in the maternal plasma; (d) to ascertain their physiological functions; and (e) to search for correlations which may exist between abnormal pregnancies and either qualitative or quantitative variation of these pregnancy-associated plasma components.

Honors and Awards: None

Publications:

1. Lee, G., and Robinson, J. C.: Agar diffusion test for serum cholinesterase typing and influence of temperature on dibucaine and fluoride numbers. J. Med. Genet. 4: 19-25, March 1967.

2. Robinson, J. C., and Lee, G.: Preparation of starch gel zymograms: Peroxide-producing enzymes and ceruloplasmin. Arch. Biochem. 120: 428-433, May 1967.

3. Robinson, J. C., Levene, C., Blumberg, B. S., and Pierce, J. E.: Serum alkaline phosphatase types in North American Indians and Negroes. J. Med. Genet. 4: 96-101, June 1967.

4. Robinson, J. C., and Goldsmith, L. A.: Genetically determined variants of serum alkaline phosphatase: A review. Vox Sang. 13: 289-307, Oct. 1967.

5. Chase, H. P., Dorsey, J., and McKhann, G. M.: The effect of malnutrition on the synthesis of a myelin lipid. Pediatrics 40: 551-559, Oct. 1967.

6. Chase, H. P., Goodman, S. I., and O'Brien, D.: Treatment of homocystinuria. Arch. Dis. Child. 42: 514-520, Oct. 1967.

Serial No. HD-LB2
1. Laboratory of Biomedical Sciences
2. Section on Developmental Enzymology
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Transsulfuration in Developing Primate Brain

Previous Serial Number: None

Principal Investigator: J. Volpe

Other Investigators: L. Laster

Cooperating Units: L. Laster, Metabolic Diseases Branch, Section on Gastroenterology, NIAMD

Man Years

Total:	19/12
Professional:	8/12
Others:	11/12

Project Description:

Objectives: The main objectives of this project are: (1) to perform a regional survey of the activity of methionine-activating enzyme and cystathionine synthase and of the level of cystathionine (as well as other amino acids) in 18-22 regions of the brain of the juvenile Rhesus monkey, (2) to determine the development of the activity of methionine-activating enzyme and cystathionine synthase and of the level of cystathionine in 16 regions of the brain of the developing Rhesus monkey, and (3) to elucidate some of the factors controlling the accumulation of cystathionine (found in uniquely high concentration only in primate brain) in primate brain.

Methods Employed: The principal methods employed are: (1) radioactive assay based on the conversion of carbon-labeled (methyl) methionine to S-adenosylmethionine and of carbon-labeled (3-position) serine to cystathionine, and (2) amino acid analysis after amino acid extraction by homogenization in acid buffer, ultracentrifugation (78,500xg) and deproteinization of supernatant. Amino acid analysis is accomplished in an automatic amino acid analyzer utilizing a continuous gradient buffer system.

Major Findings: A total of 20 brains were dissected and analyzed from animals in early fetal life to 2 to 3 years of postnatal life.

Methionine-activating enzyme activity was highest in the pituitary gland (anterior plus posterior), midline and lateral cerebellum and occipital

cortex. Relatively high levels were also found in caudate and putamen of basal ganglia. Lowest levels were found in areas rich in white matter.

Cystathionine synthase activity was highest in midline and lateral cerebellum, occipital cortex and globus pallidus. Relatively high levels were also found in the other cortical gray areas and superior and inferior colliculi. Lowest levels were found in areas rich in white matter.

No major developmental changes were noted in either enzyme in any are of brain. However, values in fetal cerebrum were among the highest at any later age.

Cystathionine was found in highest concentrations in areas rich in white matter, especially parietal and occipital white matter. However, cerebellar areas and globus pallidus, neither unusually rich in white matter, also had high levels of cystathionine. Lowest levels were in cortical gray areas. Very low levels were noted in fetal cerebrum; however, the 10 and 14-week brains had values as high as the oldest animals.

Significance to Biomedical Research and the Program of the Institute: These experiments demonstrate: (1) good correlation between some known aspects of transmethylation and activity of methionine-activating enzymes, (2) possibility that previously undefined methylation(s) are important in metabolism of cerebellum, (3) factors other than or in addition to the level of cystathionine synthase activity control the accumulation of cystathionine in brain, (4) cystathionine may be produced in areas rich in nerve cell bodies and be transported and concentrated along nerve fibres, and (5) trans-sulfuration is as active, and perhaps more so, in the early fetus as in the more mature animal.

These experiments have indirect bearing on the clinical problem of homocystinuria. The presence of high activity in the early fetus suggests that this area of metabolism is important in the early development of primate brain and that neurologically abnormal children are not likely to result from pregnancies of clinically normal homocystinuric mothers (unlike the case with children born to mothers with undetected phenylketonuria).

Proposed Course of Project: The project is now completed.

Honors and Awards: None

Publications: None

Serial No. HD-LB 3

1. Laboratory of Biomedical Sciences
2. Section on Developmental Enzymology
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Development of Cystathionine Synthase Activity in Rat Liver and Brain: Hormonal and Dietary Influences

Previous Serial Number: None

Principal Investigator: J. Volpe

Other Investigators: L. Laster

Cooperating Units: L. Laster, Metabolic Diseases Branch, Section on Gastroenterology, NIAMD

Man Years

Total:	11/12
Professional:	6/12
Others:	5/12

Project Description:

Objectives: The main objectives of this project are: (1) to determine the development of cystathionine synthase activity in the liver and brain of the developing rat, and (2) to elucidate the factors, humoral, dietary and otherwise, that influence and determine this development.

Methods Employed: The principal method employed was a radioactive assay based on the formation of L-cystathionine-3-C^{14} and L-homocysteine. Protein determinations were made by a spectrophotometric procedure.

Major Findings: In liver, cystathionine synthase activity rises 10-20 fold on the 20-21st days of gestation from barely detectable levels. A more gradual rise in activity occurs in the next 2-3 weeks of postnatal life. Experiments with an inhibitor of protein synthesis (cycloheximide) demonstrate that the fetal rise in activity is related to new enzyme protein synthesis. The rise cannot be prematurely induced by glucagon ACTH, hydrocortisone, growth hormone or thyroxine. The rise cannot be prevented by large doses of conjugated estrogens.

In brain, cystathionine synthase activity rises 5-10 fold from barely detectable levels in the term fetus to adult levels by the end of the first week of postnatal life. The rise cannot be prematurely induced by glucagon.

Adrenalectomy has no effect on the postnatal rise in either liver or brain.

Significance to Biomedical Research and the Program of the Institute: These experiments demonstrate: (1) a dramatic developmental pattern of hepatic enzyme activity and a means of studying the factors influencing and determining such activity in fetal liver, and (2) a sharp postnatal rise in brain enzyme activity at a time when neurons are undergoing rapid growth and differentiation, as well as a means of studying the factors influencing and determining such activity in mammalian brain, the latter being a still poorly understood area.

Proposed Course of Project: The project is to be continued particularly in regard to item (2) under "Objectives." The humoral factors studied in the fetus will be investigated in the postnatal liver and brain. Moreover, dietary factors, particularly pyridoxine, will also be studied.

Honors and Awards: None

Publications: None

Serial No. HD-LB4
1. Laboratory of Biomedical Sciences
2. Section on Physiological Controls
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Regulation of Liver Metabolism

Previous Serial Number: None

Principal Investigator: W. H. Glinsmann

Other Investigators: None

Cooperating Units: None

Man Years

Total:	30/12
Professional:	12/12
Others:	18/12

Project Description:

Objectives: To clarify the mechanisms involved in the control of hepatic glucose output and adaptive enzyme formation. Present experiments focus on: (1) the description of the endogenous capacity of rat liver to regulate blood glucose concentration and alter glycogen synthesis and degradation in response to alterations in circulating levels of glucose, (2) the effects of insulin and glucocorticoids on glycogen synthesis and adaptive enzyme formation, and (3) nucleotide regulation of the activity of enzymes controlling glycogen synthesis and degradation.

Methods Employed: The technique for the isolated perfusion of rat livers has been frequently used since it allows for the delineation of endogenous responses of the liver to metabolic substrates and intermediates from the effects of hormones given alone or in combination. Net changes in the functional capacity of metabolic pathways involved in gluconeogenesis and glycogen metabolism are correlated with changes in tissue concentration of metabolic intermediates and activity of rate limiting enzymes. Chemical and enzymatic assays are based on standard chromatographic, spectrophotometric and radioisotopic tracer techniques.

Major Findings: Following a study of the conditions of liver perfusion which optimally stabilize basal rates of glycogenolysis and maximize tissue uptake of oxygen, we investigated the responses of livers from normally fed animals to acute alterations in circulating glucose concentration. The perfused

livers released glucose and degraded glycogen in response to hypoglycemia and took up glucose and synthesized glycogen in the presence of continued hyperglycemia. Livers from fasted animals synthesized glycogen at lower levels of hyperglycemia than livers from fed animals. The responses were induced rapidly and were large in magnitude supporting the hypothesis that homeostatic control of hepatic glucose output in vivo is in part mediated by the direct effects of circulating glucose concentration on hepatic glucose metabolism.

Continuing our studies on the action of insulin on hepatic glycogen metabolism, we have tested the effect of the hormone on glycogen synthesis in the perfused rat liver under a variety of conditions in which glycogen was being actively synthesized or degraded. The results support our previous observation that insulin acts primarily to inhibit glycogenolysis when it is submaximally stimulated. An effect of insulin to enhance net glycogen synthesis could not be obtained. The inhibitory effect of insulin on glycogenolysis could be demonstrated in the absence of any detectable alteration in phosphorylase activity which lends additional support for our earlier conclusion that the effect of insulin to inhibit hepatic glucose output is not mediated by an action of the hormone to lower tissue levels of 3',5'-AMP.

In vivo studies in fasted rats have confirmed the observation that glucocorticoids stimulate hepatic glycogen synthesis and increase the formation of tyrosine transaminase (TT), and that these responses are largely, but not completely, inhibited by the antibiotic Actinomycin D (ACTD) which inhibits DNA-dependent synthesis of RNA. Administration of oral glucose enhanced glycogen synthesis without increasing TT activity in both fasted normal and adrenalectomized animals and ACTD continued to inhibit glycogen synthesis under these conditions.

Further examination of these responses in the isolated perfused rat liver have shown a direct effect of dexamethasone and hydrocortisone to enhance glycogen synthesis and increase TT activity. Glycogen synthesis induced by hyperglycemia in this system was, however, not altered by ACTD showing that this direct effect of glucose did not require new message formation. Since the effect of glucose loading to enhance glycogen synthesis in vivo was obtainable in adrenalectomized animals and was largely inhibited by ACTD, it was likely mediated by nonsteroidal hormones.

Acute hormonal regulation of glycogen synthesis and degradation is thought to be mediated by changes in tissue levels of the cyclic nucleotide 3',5'-AMP which in turn governs the rate of phosphorylation (and hence activity) of the enzymes phosphorylase (P) and UDPGlucose-glycogen transferase (GT). An in vitro effect of 5'-AMP to lower substrate Km's of liver P is known, but an in vivo control of liver glycogen metabolism has not been shown. It has been postulated that increases in tissue 5'-AMP may mediate glycogenolysis occurring with hypoxia. We have shown that hypoxia and direct administration of 5'-AMP to the perfusion medium markedly stimulates glycogenolysis in the isolated perfused rat liver. This is associated with a marked activation of P and inhibition of GT activities which occurs within seconds.

In studies where methylated xanthines were used to competitively inhibit 3',5'-AMP phosphodiesterase activity to discriminate between the effects of 3',5'-AMP and 5'-AMP on glycogenolysis, a direct effect of caffeine to inhibit phosphorylase activation was also seen. Caffeine concentrations above 10^{-3}M noncompetitively inhibit glycogenolysis and phosphorylase activation induced by glucagon, 3',5'-AMP and 5'-AMP, although stimulation of hepatic urea production by glucagon is enhanced by caffeine administration.

Significance to Biomedical Research and the Program of the Institute: The present studies have in large part been descriptive of the endogenous capacity of the isolated liver to regulate glucose and glycogen metabolism and respond directly to administered hormones and nucleotides since in previous in vivo studies it has not been possible to delineate clearly the effects of hormonal alterations from the effects of simultaneous changes occurring in circulating metabolites. The further development of liver perfusion methodology for these studies will be of use to other investigators interested in the study of isolated organ perfusions. The findings of direct effects of glucose concentration and glucocorticoids on hepatic glycogen metabolism and hepatic glucose output are now able to be further studied regarding mechanisms involved in their mediation. The findings of a direct action of 5'-AMP on phosphorylase and UDPGlucose-glycogen transferase activation and glycogenolysis may partially explain nonhormonal alteration in glucose metabolism (such as occurs with hypoxia). It is anticipated that extension of the present findings will help to clarify the mechanisms whereby 3',5'-AMP, an important hormonal intermediate, and glucose modulate hepatic glucose metabolism and adaptive reactions of the liver which are controlled at the level of protein synthesis.

Proposed Course of Project: Studies will continue to focus on the mechanisms which control cyclic nucleotide metabolism in liver and modify the effects of 3',5'-AMP on hepatic carbohydrate and protein turnover. The role of cyclic nucleotides in the modulation of hepatic regeneration growth will be assessed.

Honors and Awards: None

Publications:

1. Glinsmann, W. H., and Mortimore, G. E.: Influence of glucagon and 3',5'-AMP on insulin responsiveness of the perfused rat liver. Amer. J. Physiol. (In press).

Serial No. HD-LB5

1. Laboratory of Biomedical Sciences
2. Section on Physiological Controls
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Regulation of Adrenal Growth and Metabolism by ACTH

Previous Serial Number: None

Principal Investigator: R. V. Farese

Other Investigators: None

Cooperating Units: None

Man Years

Total:	20/12
Professional:	12/12
Others:	18/12

Project Description:

Objectives: The major objective of this project is the elucidation of the mechanisms whereby ACTH interacts with adrenal tissue and regulates the growth and metabolic function thereof. To this end attention has been focused on: (1) the possible role of adenosine-3',5'-monophosphate (CAMP) as an intracellular mediator for ACTH action on steroidogenesis, adrenal protein synthesis, and adrenal RNA synthesis, (2) the role of protein synthesis and a "3rd factor or protein mediator" during ACTH action on steroidogenesis.

Methods Employed: In studies concerning the role of CAMP as an intracellular mediator of ACTH action, rat adrenal sections are incubated in Krebs-Ringer bicarbonate buffer with or without ACTH or CAMP, and various parameters of adrenal metabolism are studied (e.g., corticosterone production, incorporation of labeled precursors into protein and RNA, etc.). In studies concerning the possible involvement of a protein mediator during ACTH action on steroidogenesis, extracts from control and ACTH-stimulated adrenals are assayed for their ability to regulate cholesterol side chain cleavage (the rate-limiting step in the steroidogenic biosynthetic pathway) by incubating with adrenal mitochondria prelabeled with cholesterol-26-^{14}C, and isolating ^{14}C-isocaproic acid resulting from such cleavage.

Major Findings: After incubation of adrenal tissue in vitro with ACTH or CAMP for 30 minutes, it was found that heightened rates of steroidogenesis persist without diminution for 30-40 minutes, and then decay with a half-life

of approximately 25 minutes. This effect persists despite removal of the stimulator (ACTH or CAMP) from the incubation media, and despite addition of cycloheximide or chloramphenicol to the incubation media to block any new inductive action of residual stimulator (note--these inhibitors completely block the effects of ACTH and CAMP on steroidogenesis if added at time zero with the stimulator). The parallelism between the response, persistence and decay of ACTH-induced and CAMP-induced steroidogenesis offers more evidence suggesting that a common mechanism is involved, and that CAMP may be the intracellular mediator of this effect of ACTH. Furthermore, the persistence of the steroidogenic effect under the experimental conditions described strongly suggests that a third factor, presumably a steroidogenic protein, has been induced and continues to operate on steroidogenesis even after new induction of this factor is blocked.

In previous studies by numerous investigators, ACTH and CAMP had been found to inhibit incorporation of precursors into protein and RNA. Thus, despite the well-known fact that ACTH dramatically stimulates adrenal RNA and protein synthesis after administration in vivo, it had not been possible to study the tropic action of this hormone in vitro. In our studies we have found that the observed inhibitory effect of ACTH and CAMP on precursor incorporation are largely artifactual owing to the accumulation of steroids in the incubation medium. If steroid production is inhibited by aminoglutethimide (elipten), ACTH is found to enhance incorporation of H^3-leucine into protein and ^{14}C-orotic acid into RNA. Under these conditions, CAMP also enhances ^3H-leucine incorporation, but the effects on ^{14}C-orotic acid incorporation are more complex, viz., inhibition of low doses, and a stimulatory component (but never higher than baseline) at higher doses. The inhibitory effects of low doses of CAMP appear to be due to metabolism to 5'-AMP since the inhibition is blocked by caffeine, and since 5'-AMP itself is markedly inhibitory at all concentrations employed. The effects of ACTH on ^{14}C-orotic acid incorporation into RNA are not blocked by cycloheximide, and the effects of ACTH (and CAMP) on ^3H-leucine incorporation into protein are not blocked by actinomycin D. It is suggested that ACTH and CAMP may regulate adrenal protein synthesis at the translation level and this may be of importance in the steroidogenic effects of these substances. Furthermore, our findings are compatible with the possibility that CAMP may mediate this effect of ACTH on adrenal protein synthesis. Unfortunately, the results with ^{14}C-orotic acid incorporation into RNA do not shed further light on the possibility that CAMP may be involved in the regulation of adrenal RNA synthesis by ACTH.

Our studies concerning the role of protein synthesis and a protein mediator in the regulation of steroidogenesis by ACTH have been extended during the past year. Following stimulation by ACTH in vivo, dialyzed 60,000 xg-supernatant derived from adrenal homogenates has been found to enhance cholesterol side chain cleavage (relative to control supernatant) when incubated in our assay system. The change in supernatant activity is rapidly (2-5 minutes) induced by ACTH or ether stress, and it decays rapidly in vivo after injection of cycloheximide. There appears to be reasonably good parallelism between the changes in supernatant activity (assayed in vitro) and the changes in steroid production (occurring in vivo), and these results are compatible with the possibility that ACTH induces the synthesis of a rapidly-turning over steroidogenic protein.

Significance to Biomedical Research and the Program of the Institute: Our studies have enabled us to study some of the early steps in the control of adrenal growth and function by ACTH. Hopefully, as the present findings are enlarged upon and extended, it may be possible to define and examine more fully the intermediate steps which occur between the initial interaction of ACTH with adrenal tissue and the gross changes in growth and function which ensue. Using the ACTH-adrenal system as a useful model (in which rapid and dramatic changes can be effected and studied), it is hoped that findings from this system will have a more general applicability and shed more light on the complex mechanisms involved in the growth, development, and metabolic function of other tissues as well.

Proposed Course of Project: This project will continue during the next year.

Honors and Awards: None

Publications:

1. Farese, R. V.: ACTH-Induced changes in the steroidogenic activity of cell-free preparations of the rat adrenal. In Margoulies, M. (Ed.): Protein and Polypeptide Hormones. Amsterdam, Exerpta Medica Foundation, 1968, pp. 181-184.

2. Farese, R. V.: ACTH, protein synthesis, and steroidogenesis. Clinical Research 16: 265, 1968.

NICHD Annual Report
July 1, 1967 through June 30, 1968

Children's Diagnostic and Study Branch

Scientific Activities: The Children's Diagnostic and Study Branch is an outpatient facility where research is conducted in the fields of medicine, biology, behavioral sciences, education, and epidemiology concerning various handicapping conditions such as mental retardation, learning disabilities, motor defects, and sensory deficits. In addition, diagnostic studies are carried out on mentally retarded or physically incapacitated children using a multispecialty approach. Diagnostic studies are restricted to dependents of military personnel. A number of research projects are incorporated within the context of our diagnostic and evaluative activities. However, the major bulk of our research projects involve subjects who are not included in our diagnostic studies. These subjects include newborn infants at the National Naval Medical Center nursery, preschool and school age children in Washington, D. C. and nearby counties as well as patients in institutions for the mentally retarded.

The research activities of the Branch are determined by the interests of the staff. Because of the nature of our activities, the Branch attracts scientists from a number of disciplines and makes use of the knowledge, expertise, and technology in a number of disciplines. Hence, our research program includes studies in immunogenetics, cytogenetics, population genetics, speech and language development, maternal-infant interaction, counseling, neurology and others.

For the past year the clinic evaluated 94 children including 31 who had been seen previously by the staff. In addition, a number of patients were referred from the Bethesda Naval Hospital and other military hospitals to the Cytogenetics Unit of our Branch for chromosomal studies. As a result of our clinical and cytogenetic activities we have encountered a number of interesting patients such as a child with congenital sensory neuropathy or congenital anesthesia to pain, touch, and temperature who, in spite of his handicap, is intellectually and emotionally normal; a 4½ year old mentally retarded child with monosomy G in the blood, skin, and bone marrow; an infant with Apert's syndrome and associated chromosomal anomaly which is believed to be the first patient with this diagnosis to manifest cytogenetic aberration; a patient with deletion of the short arm of an X chromosome with phenotypic appearance of Turner's syndrome and a child with phenotypic and dermatoglyphic characteristics of Down's syndrome (mongolism) but with a normal karyotype in the peripheral blood.

The ultimate goal of the Children's Diagnostic and Study Branch is prevention of mental retardation and other handicapping conditions which impair achievement of a child's potential. The key to prevention of mental retardation is an adequate knowledge of its causes. A better understanding of etiology is mandatory before one can develop meaningful preventive and therapeutic measures. It is, therefore, essential that a diagnosis is made so that counseling and prognosis can be formulated. To this end various investigations are being conducted or in various stages of planning within the Branch. Some

of these studies will require inpatient facilities for satisfactory behavioral and biomedical observations and analysis.

Other Professional Activities: The Cytogenetics Unit was transferred to the Children's Diagnostic and Study Branch from the Laboratory of Biomedical Sciences. Neurological evaluation and electroencephalographic interpretation is being carried out by the Behavioral Biology Branch, NICHD.

Dr. John Lynch, pediatrician with the National Naval Medical Center, spent two months in the clinic as guest investigator. In this capacity he participated in the evaluation of patients and worked with various members of our staff. The Chief of Pediatrics at the National Naval Medical Center has expressed interest to continue this type of program to include other pediatricians on his staff.

The staff is composed of 19 individuals (13 professional including a consultant in child psychiatry, 2 technical, and 4 secretarial/clerical).

Dr. Louie Linarelli took the NIH sponsored course on Radioisotopes. Dr. Felix de la Cruz attended a postgraduate course in Pediatric Neurology which was sponsored by the Department of Neurology, Columbia University College of Physicians and Surgeons in New York.

Various members of the staff organized, participated, or acted as chairmen of various scientific meetings, both on a local and national level. In addition, some members of the staff were involved in the development of a comprehensive plan on mental retardation for the District of Columbia.

The clinic is still faced with the problem of depending upon outside facilities in the performance of our routine activities such as neurological evaluation, EEG tracing, and biochemical assessment of biological specimens. Also, performance of research on non-military subjects is difficult to accomplish in view of the restriction imposed on the staff of not being allowed to evaluate civilian children in our facilities. We have been visiting public and private schools, homes, residential facilities and hospitals in order to obtain research data, or scheduling patients to come to NIH on weekends where clinical assessment is carried out in a controlled, i.e. sound proofed booth, setting.

Serial No. HD-MR9(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cytogenetic Aspects of Mental Retardation

Previous Serial Number: HD-MR9

Principal Investigator: Matti S. Al-Aish, M.A., Ph.D., Research Cytogeneticist

Other Investigators: Gordon Mella, M.D., Department of Pediatrics, National Naval Medical Center

Cooperating Unit: National Naval Medical Center, Bethesda, Maryland

Man Years

Total	:	0.45
Professional	:	0.45
Other	:	None

Project Description:

Objectives: This project is designed to: (1) conduct a complete chromosome analysis of mentally retarded children and to perform thorough chromosome morphology studies to determine the cytogenetic basis of certain forms of mental retardation, (2) collaborate with others in related scientific fields such as biochemistry and enzymology in the investigation of chromosome deletion, trisomies, or monosomies. Chromosome mapping is the ultimate objective of this combined cytogenetic and biochemical approach.

Methods Employed: Chromatin body studies are done routinely from buccal smears of all patients. Short term tissue cultures of peripheral blood is our primary source for patients' chromosomes. Cultures of bone marrow and skin are also used for more extensive cytogenetic evaluation. Autoradiographs for chromosome identification and drumstick preparations for sex chromosome studies in females were done in special cases.

Major Findings: (1) The results of studies on the cytogenetic basis for the De Lange syndrome were presented by Dr. Al-Aish at The Mental Retardation Research Seminars. (2) An unusual case of Turner syndrome (45/XO, fragments, rings). (3) A first complete autosomal monosomy was found in a mentally retarded girl. The aneuploidly involves a small acrocentric chromosome in the

21-22 group and tentatively identified as chromosome number 21. Mosaicism and translocation were ruled out. These findings were presented by Dr. F. de la Cruz at the First Congress of the International Association for the Scientific Study of Mental Deficiency which was held in Montpellier, France on September 12-20, 1967.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project will increase our knowledge of the cytogenetic correlates of abnormal development. The project is a vital part of the Institute's interdisciplinary study of mental retardation.

Proposed Course of Project: The screening of mentally retarded children for chromosomal and dermatoglyphic abnormalities will continue. Further appropriate studies of the patient such as the patient with autosomal monosomy will include further cytogenetic investigation of the proband's mother and her identical twin sister. Additional evaluation of enzymes and other proteins will be carried out to detect gene loci linked to the missing chromosome for future mapping of chromosome number 21.

Honors and Awards: Dr. Al-Aish has been invited to deliver the autosomal monosomy findings at the XII International Congress of Genetics in Tokyo, Japan on August 19-28, 1968.

Publications: Al-Aish, M., de la Cruz, F, Goldsmith, L.A., Volpe, J., Mella, G., and Robinson, J.C. Autosomal Monosomy in Man. Complete Monosomy G (21-22) in a Four-and-one-half-year-old Mentally Retarded Girl. New England Journal of Medicine 277: 777-784, Oct. 1967.

Serial No. HD-MR10/11(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Morphology Studies on Normal and Abnormal Human Chromosomes

Previous Serial Number: HD-MR10/11

Principal Investigator: Matti S. Al-Aish, M.A., Ph.D., Research Cytogeneticist

Other Investigators: None

Cooperating Units: National Naval Medical Center, Bethesda, Maryland

Man Years

Total	:	0.5
Professional	:	0.5
Other	:	None

Project Description:

Objectives: The objective of this project is to develop methods for the more precise identification of individual chromosomes. The morphology of metaphase chromosomes is the basis of the present day diagnostic procedure of cytogenetics. Many of the chromosomes do not have unique morphology and must, therefore, be placed in groups for classification. A better correlation of specific chromosome aberrations with specific clinical syndromes can be expected when methods have been established for the unequivocal identification of each chromosome.

Methods Employed: A combination of physical, chemical and enzymological methods will be employed. More specifically, this objective can be pursued by treating the chromosomes with enzymes (e.g., proteases and nucleases), by radioactive labeling techniques, and chemicals such as 5-Bromodeoxyuridine and Ca-free media. Another approach is the accurate relative measurements of each chromosome, its arm ratio, and centromeric index. In addition, a differential interference analysis of each chromosome will be done.

Major Findings: Chromosome measurements, arm ratios, and centromeric indices of a large number of chromosomes conclusively proved that group C (X-6-12) of the human karyotype can be subdivided into four subgroups. A manuscript describing this new human chromosome classification and karyotyping is being prepared.

Significance to Biomedical Research and the Program of the Institute: The precise identification of human chromosome is the foundation of human genetic studies. Without the accurate identification of human chromosomes, genetic mapping of chromosomes, the biochemical basis of inheritance, and the description of genetic syndromes would be impossible.

Proposed Course of Project: More studies will be conducted on mitotic chromosomes with emphasis on primary and secondary constriction. Morphological studies on human chromosome behavior during the meiotic division will also be conducted.

Honors and Awards: None

Publications: None

Serial No. HD-CD1(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Clinical Cytogenetics and Cytogenetic Aspects of Mental Retardation

Previous Serial Number: None

Principal Investigator: Ronald P. Bachman, M.D., Clinical Associate

Other Investigators: None

Cooperating Units: National Naval Medical Center, Bethesda, Maryland

Man Years

Total	:	0.25
Professional	:	0.25
Other	:	None

Project Description:

Objectives: To determine the frequency of chromosomal, dermatoglyphics and genetic abnormalities among children that are evaluated at the Children's Diagnostic and Study Branch.

Methods Employed: Two mentally retarded or physically handicapped children are evaluated weekly with chromosomal analysis, buccal smears, dermatoglyphics and other indicated studies. Family studies are performed when indicated. Chromosomal analysis is done using a modification of Moorhead's procedure. Buccal smear is obtained using modification of Barr's procedure. Dermatoglyphics are observed visually with a hand lens and then recorded in ink.

Major Findings: As a result of routine cytogenetic studies on a heterogeneous group of children with varying complaints the following patients were encountered: 1) Patient with congenital hypoplastic anemia. This patient was referred from the Department of Pediatrics, Bethesda Naval Hospital with the Chief Complaint of Hemolytic Anemia. Cytogenetic studies disclosed multiple breaks and aneuploid cells. 2) A case of deleted X syndrome is reported elsewhere. Briefly the study indicates that the gene (s) responsible for the phenotypic manifestations of Turner's syndrome are located on the small arm of X chromosomes. 3) Cytogenetic abnormality which has been reported

for the first time on an infant with Apert's syndrome. This study is in progress but present findings indicate 2/B translocation. 4) A case of congenital multiple malformations with a B/C translocation. 5) A case of D/D translocation. 6) A case of Down's Syndrome with borderline intelligence who has phenotypic manifestations of Down's Syndrome but with normal karyotype in the peripheral blood.

Significance to Biomedical Research and the Program of the Institute: This will increase our knowledge of the cytogenetic correlates of abnormal development. A number of cytogenetic aberrations would have been missed if cytogenetic studies were not a routine part of developmental studies at the Children's Diagnostic and Study Branch.

Proposed Course of Project: Some of the conditions will be studied in more detail. Screening of children with mental retardation and congenital malformation for cytogenetic abnormalities will continue.

Honors and Awards: None

Publications: None. The patient with the deleted X syndrome was presented at the Third Annual Meeting of the Commissioned Officers Association of the Public Health Service which was held in San Francisco, California in March 1968.

Serial No. HD-CD2(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cytogenetic and Dermatoglyphic Analysis of the De Lange Syndrome

Previous Serial Number: None

Principal Investigators: Ronald P. Bachman, M.D., Clinical Associate
Joseph J. Volpe, M.D., Clinical Associate
Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development
Adam P. Matheny, Ph.D.
Department of Pediatrics, University of Louisville
Louisville, Kentucky

Other Investigator: Michael Rostafinsky, M.D.
Lynchburg Training School and Hospital

Cooperating Units: Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development
Lynchburg Training School and Hospital
Colony, Virginia

Man Years

Total	:	0.13
Professional	:	0.13
Other	:	None

Project Description:

Objectives: The purpose of the study is to establish the chromosomal patterns in a group of children who have the clinical manifestations of the De Lange Syndrome. Dermatoglyphic analysis would also be done. It is planned to study the dermatoglyphics of all parents of the children. Any of the patients with chromosomal abnormalities would have family studies done (chromosomal). Pedigrees, history and physical examination, developmental assessment and photographs would be included in the study.

Methods Employed: Ten cc of blood would be drawn into a heparinized syringe. This blood will be processed by a modified Moorehead procedure. Buccal smears

would be taken and processed by a modified Barr procedure. Dermatoglyphics will be evaluated with a hand lens and then recorded in ink. Developmental assessment will be done.

Major Findings: Study in Progress.

Significance to Biomedical Research and the Program of the Institute: The genetics of this Syndrome has not been established. A number of mechanisms have been proposed including a cytogenetic abnormality, a dominant gene or a recessive gene type of transmission. It is hoped that this study will help establish the genetics of this Syndrome.

Proposed Course of Project: Any significant findings will be studied in greater detail.

Honors and Awards: None

Publications: None

Serial No. HD-CD3(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Effects of Irradiation on the Induction of Chromosomal Abnormalities

Previous Serial Number: None

Principal Investigator: Ronald P. Bachman, M.D., Clinical Associate

Other Investigator: Francis Depenbusch, M.D.
Charleston Naval Hospital

Cooperating Unit: Charleston Naval Hospital
Charleston, Virginia

Man Years

Total	:	0.21
Professional	:	0.21
Other	:	None

Project Description:

Objectives: The effects of gamma irradiation was studied on human subjects. Peripheral blood was obtained from men who were aboard nuclear submarines, and were exposed to greater than maximal permissible doses of gamma irradiation. This was determined by reading their film badges after a cruise (3-6 months). The allowable level of irradiation is about .3 rem per week during the cruise. Dosimetry was such that the exact amount of gamma irradiation could not be determined. It was only known that greater than the allowable dose was received.

The chromosomes from the peripheral blood were studied for evidence of cytogenetic aberrations and this was compared to a group matched for age and sex who had not received any irradiation. None of the subjects had had recent exposure to diagnostic irradiation.

Methods Employed: Peripheral blood was obtained and inoculated into NIH culture media and then air freighted to our laboratory from Charleston, North Carolina. The specimen was then processed according to a modified Moorhead procedure. A total of 12 subjects were studied--6 controls and 6

subjects who had received the excessive irradiation. At least 20 cells were studied on each patient and these cells were examined for breaks, gaps, deletions, aneuploidy, polyploidy, and triradial forms. The subjects were healthy males ages 18-30 who were members of nuclear submarine crews.

Major Findings: In the group studied (subjects) there were 11.6% abnormal chromosomes and in the controls there were 13% abnormal chromosomes. This difference was not significant.

There were more aneuploid cells in the subjects (16.3% versus 13% in controls) but this was also not significant.

Significance to Biomedical Research and the Program of the Institute: It has been shown that relatively low levels of irradiation can cause transient and permanent damage to the genetic material. The present study was done to see if the allowable levels for gamma irradiation were safe. The number of subjects in this study was small, but it appears that even if one only slightly exceeds the MPD that there is no genetic damage.

Proposed Course of Project: The study has been terminated.

Honors and Awards: None

Publications: None

Serial No. HD-CD4(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Study of the Deleted X Syndrome

Previous Serial Number: None

Principal Investigator: Ronald P. Bachman, M.D., Clinical Associate

Other Investigators: None

Cooperating Units: Department of Medicine, National Naval Medical Center, Bethesda, Maryland

Man Years

Total	:	0.17
Professional	:	0.17
Other	:	None

Project Description:

Objectives: A patient with deletion of the short arms of one of her X chromosomes has been studied in detail. There have been only five previous cases described in the literature. This patient and her family have been studied. Genetic studies plus correlation of the phenotype and karyotype have revealed that the genes which control the congenital malformations of Turner's Syndrome are located on the short arms of the X chromosome.

Methods Employed: Standard genetic laboratory procedures have been utilized. These include karyotype study, buccal smear, dermatoglyphics, Xg^a blood grouping, color vision studies, pedigree analysis, and glucose-6-phosphate dehydrogenase studies.

Major Findings: Patients with short arm deletion of the X chromosome are phenotypically identical to patients with Turner's Syndrome.

Significance to Biomedical Research and the Program of the Institute: The gene or genes which control short stature and the other malformations of Turner's Syndrome are located on the short arm of the X chromosome.

Proposed Course of Project: The data have been presented at the Third Annual Commissioned Officers Association of the Public Health Service at San Francisco, California. A manuscript describing the study of this case is in preparation.

Honors and Awards: None

Publications: None

Serial No. MD-CD5(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Speaking Fundamental Frequency (SFF) Characteristics of Children With Mongolism

Previous Serial Number: None

Principal Investigators: Bernd Weinberg, Ph.D., Research Fellow
Oral and Pharyngeal Development Section
National Institute of Dental Research
Marsha Zlatin Hess, M.A., Speech Pathologist

Other Investigators: None

Cooperating Unit: Oral and Pharyngeal Development Section
National Institute of Dental Research

Man Years:

Total	:	0.30
Professional	:	0.15 (1 person)
		0.15 (1 person)
Other	:	None

Project Description:

Objectives: The general purpose of this investigation will be to define the speaking fundamental frequency characteristics of young children with mongolism and to compare such characteristics to "normal" children paired on the basis of age and sex. The primary objective of this investigation will be to provide useful empirical information to test the clinical observation and hypothesis that children with mongolism characteristically speak with lower speaking fundamental frequencies compared with "normal" children of comparable age and sex.

Methods Employed: Subjects: 40 Children with Mongolism
10 males: 5-0 to 5-11/12 years
10 females: 5-0 to 5-11/12 years
10 males: 6-0 to 6-11/12 years
10 females: 6-0 to 6-11/12 years

40 Normal Control Children Matched for Age and Sex

Subjects Secured From: Children's Diagnostic and Study Branch, NICHD
Montgomery County Association for Retarded Children (MARC)
St. Johns Development Center
Drew Smith Training Center
Hope Day Care Center, Inc.
Prince George's County Retarded Day Care Center, Inc.
Children's Hospital
Primary Day School

Speech samples will be obtained from 80 subjects (40 children with mongolism and 40 controls). All subjects must have normal hearing for speech and be in adequate physical condition to participate in the study. Data concerning chronological age, sex, height, weight and I.Q. will be obtained at the time of the study.

Speech recordings will be made in a quiet room using high quality tape recording instrumentation (Ampex 601 and Altec M-45 microphone system).

Thirty seconds of subjects' verbalization will be analyzed for fundamental frequency measures (mean, standard deviation, and range of means) by the Fundamental Frequency Indicator. This instrumentation samples the signal period every 33 msec. and Period data are transferred to IBM punch cards.

Major Findings: Study in progress.

Significance to Biomedical Research and the Program of the Institute: Studies of average speaking fundamental frequency have been considered valuable with respect to demonstrating changes in the fundamental frequency of the voice as a function of growth, development, and aging. These studies have begun to provide normative data against which pitch abnormalities may be compared. There is a definite need for continuing research, not only for normative data, but also to specify the acoustic correlates of vocal abnormalities in the speech of "atypical" children such as those with mongolism and to attempt to correlate these with anatomic and physiological variables. This information should provide additional data concerning developmental progression of children with mongolism and possibly be of assistance in differential diagnosis of speech characteristics.

Proposed Course of Project: The present investigation should be considered as a preliminary study which will provide empirical acoustic data to support or reject a widely accepted clinical observation concerning one attribute of voice quality in children with mongolism. If the hypothesis is accepted that mongoloid children do characteristically speak in "low pitched voices", further studies will be proposed to define anatomic and physiological factors responsible for these perceptual and acoustic findings.

Honors and Awards: None Publications: None

Serial No. HD-CD6(c)

1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Special Studies on a Patient with Congenital Sensory Neuropathy

Previous Serial Number: None

Principal Investigator: Louie G. Linarelli, M.D., Clinical Associate

Other Investigators: James W. Prichard, M.D., Medical Officer, Behavioral Biology Branch, National Institute of Child Health and Human Development
David Symmes, Ph.D., Research Psychologist, Behavioral Biology Branch, National Institute of Child Health and Human Development

Cooperating Units: National Naval Medical Center, Bethesda, Maryland
Departments of Pediatrics, Radiology and Pediatric Surgery, Johns Hopkins Hospital, Baltimore, Maryland
Medical Branch, National Institute of Neurological Diseases and Blindness
Oral Medicine and Surgery Branch, National Institute of Dental Research

Man Years

Total	:	0.08
Professional	:	0.08
Other	:	None

Project Description:

Objectives: These studies were performed to diagnose and better define the condition known as congenital sensory neuropathy. Studies were required to differentiate between the following: a) congenital sensory neuropathy b) congenital indifference to pain c) hereditary sensory radicular neuropathy d) familial dysautonomia e) familial sensory neuropathy with anhidrosis. There has been only one case in the literature of congenital neuropathy that has been studied in any detail and we have performed further pertinent studies on our patient.

Methods Employed:

a) Neurological and other physical tests
   Histamine 1:1000 skin test (to demonstrate axon reflex)
   Mecholyl 2.5% ophthalmological response
   Ammonia smelling test (Trigeminal pain response)
   Quinine and dextrose taste test
   Sweat test by iontophoresis (rule out anhidrosis)
   Cold pressure response (blood pressure response to cold pain)
   Auditory, visual and somatic evoke potentials
   EEG
   EMG
   Motor and sensory conduction studies
   Spinal tap analysis
   Rectal manometry study of internal and external sphincter with balloons and EMG

b) Lab analysis
   Serum - electrolytes, Ca, alkaline phosphatase, uric acid, total protein, T_3 resin uptake, amino acids, cytogenetics
   Urine uric acid, VMA

c) Biopsies
   Skin from finger tips and leg, muscle and sural nerve

d) X-rays
   Skull, chest, bone age and cineradiography of pharynx and esophagus

Major Findings: These studies describe a child with sporadic congenital sensory neuropathy. He has absence of sensation to pain, temperature and touch. To date the buccal and skin biopsies show absence of sensory nerve endings. (Presently obtaining further neuropathologic consultation). He lacks somatic evoke potential response but he has normal visual and auditory evoke response. Motor nerve conduction is normal but sensory conduction is impaired. EEG and EMG are normal. Unlike familial dysautonomia, he has normal cineradiologic studies of his pharynx and esophagus. Rectal manometry leads us to believe that he has visceral sensation since he showed discomfort with balloon inflation. The internal sphincter is normal but his external sphincter is impaired. Smell and taste may show deficits but will need further testing at appropriate age. The other studies (refer to Methods Employed) better delineated and clarified congenital sensory neuropathy from the other conditions mentioned in the Objectives category in this report.

Significance to Biomedical Research and the Program of the Institute:
The significance of this case resides in better describing this neurological entity and in relating these findings to childhood development. The existence of patients with generalized absence of sensation indicates that the body and intellect can develop without an organized peripheral sensory nervous system.

Proposed Course of Project: None

Honors and Awards: None Publications: None

Serial No. HD-CD7(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: A Serological and Hematological Genetic Survey of Chamorros in Guam

Previous Serial Number: NDB (CF) - 62 E 958

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigator: Manuel T. Cruz
National Institute of Neurological Diseases and Blindness

Cooperating Unit: National Institute of Neurological Diseases and Blindness
Agana, Guam

Man Years

Total	:	0.10
Professional	:	0.10
Other	:	None

Project Description:

Objectives: The objectives of this study are: 1) to obtain data on the distribution of the ABO, MNS, Rh, Kell, Duffy, Diego and other blood groups among the Guamanians, 2) to determine the haptoglobin frequencies in the same population, and 3) to relate the serological results with the available historical data in an effort to estimate the magnitude of Spanish, Filipino and Mexican contributions to the genetic structure of the present hybrid Chamorro population.

Methods Employed: Over three hundred specimens have been collected from five Guamanian villages. In addition to the blood grouping studies, a portion of the red cells were hemolyzed for hemoglobin investigations. Serum protein electrophoresis was also performed for the determination of the frequencies of the various haptoglobin types among the Chamorros.

Major Findings: The outstanding points of this study are: the absence of A2 and the very low frequencies of Rh negative, Fy^{a-} and Kell positive phenotypes and the presence and relatively high frequency of D_i^a. The high

gene frequency of P_2 reported earlier among the Saipan Chamorros was also shown in the present study.

The results of the blood group frequency distribution discussed in relation to the historical reports suggest that the present-day Chamorro population resembles the Filipino distribution to a greater degree than the Spanish or Mexican. Whether the similarities between Filipino and Chamorro distributions are the result of interbreeding or a reflection of a common ancestry between Filipinos or Chamorros cannot be answered by this type of investigation.

Significance to Biomedical Research and the Program of the Institute: This project contributed to the overall understanding of the genetic origin of the Chamorros of Guam.

Proposed Course of Project: None. This project has been completed.

Honors and Awards: None

Publications: Plato, C. C., and Cruz, M.T. Blood Group and Haptoglobin Frequencies of the Chamorros of Guam. Amer. J. Human Genet. 19: 722-731, 1967.

Serial No. HD-CD8(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Dermatoglyphic Evaluation of the Trukese

Previous Serial Number: NDB (CF) - 63 E 1092

Principal Investigators: Chris C. Plato, M.S., Geneticist
Jerry D. Niswander, D.D.S.
National Institute of Dental Research

Other Investigators: None

Cooperating Unit: National Institute of Dental Research, Human Genetics Branch

Man Years

Total	:	0.02
Professional	:	0.02
Other	:	None

Project Description:

Objectives: The objectives of the project in its present form are: (1) to obtain additional genetic information regarding the Trukese and to use it, along with blood group data, to estimate the relationships between the various isolates in this geographic area; and (2) to determine whether isolates with common high values of blood sugar and uric acid also have similar dermatoglyphic patterns. From earlier studies it was found that the people of Micronesia have very high values of uric acid and blood sugar. These same high values were found in other populations including South American Indians. Since the dermatoglyphics of these isolates are available this comparison was deemed valuable.

Methods Employed: Finger prints and palmprints were collected by the standard ink method from seventy Trukese and have been evaluated.

Major Findings: The dermatoglyphic patterns of the Trukese are in general similar to those reported for other parts of Micronesia and the Oriental populations for which available information exists. The fingerprints demonstrated a fairly high frequency of whorls and a marked deficiency of

arches. The palmar mainlines have in general a more transverse direction than those of Caucasian populations as indicated by both the individual line terminations as well as the model type index. The palms show a high frequency of patterns in the hypothenar region.

Significance to Biomedical Research and the Program of the Institute: This project was undertaken for two reasons: (1) to correlate fingerprint patterns with the neurological diseases found in these populations; and (2) to define the dermatoglyphics of these populations and compare them to those of other isolates.

Dermatoglyphics, like blood groups, are widely used as genetic markers as well as means of evaluating the relationship of genetic isolates. Since dermatoglyphics (finger as well as palmprints) are controlled by more than one pair of genes, their distributions are less vulnerable to genetic drift and isolation than the blood groups which are controlled by single genes.

Proposed Course: Final evaluations have been made and this project has been completed.

Honors and Awards: None

Publications: Plato, C.C. and Niswander, J.D. "Dermatoglyphics of the Trukese of Micronesia." Human Biology 39: 2, 176-181, 1967.

Serial No. HD-CD9(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Dermatoglyphics of the Islanders of Tongariki in New Herbides

Previous Serial Number: None

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigator: Carleton Gajdusek, M.D.
Special Chronic Disease Studies

Cooperating Units: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Blindness

Man Years

Total	:	0.05
Professional	:	0.05
Other	:	None

Project Description:

Objectives: To establish the finger and palm print pattern frequencies of this isolated population where genetic data have been very sparse.

Methods Employed: Eighty-five sets of finger and palm prints were collected through standard fingerprinting methods. The samples were selected at random and included both male and female subjects.

Major Findings: Incomplete; study is still in progress. The prints were read and the results have been recorded. However, the statistical analysis and evaluation of the results have not been done yet.

Significance to Biomedical Research and the Program of the Institute: It represents continuation of our effort to study and identify as many genetic markers as possible among the rapidly westernized aboriginal populations. These groups are mainly subjected to the pressures from natural selection and influenced to a lesser extent by drugs, infections and other environmental factors found in more civilized populations.

Proposed Course of Project: Our next step will be to perform the statistical tests, interpret the results and prepare a manuscript.

Honors and Awards: None

Publications: None

Serial No. HD-CD10(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Dermatoglyphics of the Kukukuku and Fore Peoples of New Guinea

Previous Serial Number: None

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: Carlton Gajdusek, M.D.
Special Chronic Disease Studies
Richard Sorenson, M.S.
Special Chronic Disease Studies

Cooperating Units: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Blindness

Man Years

Total	:	0.05
Professional	:	0.05
Other	:	None

Project Description

Objectives: To study the palmar and finger dermal configurations of these isolated aborigines who up until recently practiced cannibalism. This is in line with the objective of obtaining as much genetic information from the various aboriginal populations around the world prior to the introduction of western influence.

Methods Employed: Seventy-seven sets of finger and palm prints have been collected. Currently, Richard Sorenson is in the field (bush) collecting more data.

Major Findings: Study is still in progress.

Significance to Biomedical Research and the Program of the Institute: In addition to the aforementioned interest in the study of these aboriginal populations prior to their being influenced by western civilization, this group offers another incentive. This is the presence of a unique disease, Kuru, found only in this region.

Proposed Course of Project: To complete the collection of the data and then proceed to their evaluation and the statistical analysis and discussion of the results.

Honrs and Awards: None

Publications: None

Serial No. HD-CD11(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Dermatoglyphics of the Micronesians

Previous Serial Number: NDB (CF) - 67 E 1493

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigator: D. Carleton Gajdusek, M.D.
Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Blindness

Cooperating Unit: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Blindness

Man Years

Total	:	0.15
Professional	:	0.15
Other	:	None

Project Description:

Objectives: 1) The purpose of this project is to further define the genetic composition of the people of Micronesia, through complete dermatoglyphic studies, 2) To review the literature pertaining to the worldwide distribution of the various dermatoglyphic patterns, and 3) To discuss the significance of dermatoglyphics as a criterion for genetic relationship among different populations.

Methods Employed: The dermatoglyphic data were collected in conjunction with previous serological and epidemiological investigations in the area. Eight hundred sets of finger and palm prints from all the atolls of the district of Yap are presently being analyzed for the determination of the frequencies of the various finger and palm patterns and configurations.

Major Findings: This project is still in progress.

Significance to Biomedical Research and the Program of the Institute: A genetic identification of a population is necessary in order to facilitate and explain some of the results of the medical and neurological surveys by establishing certain patterns of gene migration or genetic relationships.

Proposed Course of Project: To continue the study of the prints and to perform statistical analysis.

Honors and Awards: None

Publications: None

Serial No. HD-CD12(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Down's Syndrome Among the Whites and Negroes

Previous Serial Number: None

Principal Investigators: C. C. Plato, M.S., Geneticist
Felix de la Cruz, M.D., M.P.H., Chief

Other Investigators: Matti Al-Aish, M.A., Ph.D., Research Cytogeneticist
Ronald Bachman, M.D., Clinical Associate

Cooperating Units: None

Man Years

Total	:	0.27
Professional	:	0.27
Other	:	None

Project Description:

Objectives: (1) To establish accurate incidence rates among the newborn and stillborn infants for Down's Syndrome among the whites and Negroes. (2) To study cytogenetically all identified cases of Down's Syndrome. (3) To determine the proportion of the different types of Down's Syndrome (Trisomy 21, translocation, mosaicism) among the whites and Negroes. (4) To perform serological and dermatoglyphic comparisons between the patients with the various types of Down's Syndrome and their respective controls. (5) To prepare and store serum and/or plasma, saliva, red blood cells for future hematological, immunological, virological and biochemical studies.

Methods Employed: This is a prospective study which will include physical and clinical examination of all babies born at the eight major hospitals of the Hampton Roads area of Virginia, which includes the cities of Norfolk, Hampton, Newport News, Portsmouth, Virginia Beach and Chesapeake. The following procedures will be undertaken on children diagnosed or suspected of having Down's Syndrome: (1) Selection of an appropriate control; the control will be the next birth (in the same hospital) who will be of the same sex and racial descent as the subject. (2) Collection of blood and dermatoglyphic specimens from both the patient and his/her respective control. (3) General

anthropometric evaluation of both patients and their respective controls and (4) Laboratory analysis and evaluation of the collected specimens. Detailed methodology is presented elsewhere.

Major Findings: Project is still in its initial stages.

Significance to Biomedical Research and the Program of the Institute: (1) To incorporate the information to be collected from the clinical epidemiological, genetic, cytological, serological and other studies in effort to establish more accurate empirical risks for genetic counseling purposes and (2) to obtain further information which might assist us and other investigators in the elucidation of the etiology of this syndrome.

Proposed Course of Project: Most of the planning of the project has been completed. We are now in the process of making arrangements with the individual hospitals for the examination of all newborn and collection of data.

Honors and Awards: None

Publications: None

Serial No. HD-CD13(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Fingerprints of the Yapese

Previous Serial Number: None

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigator: J. Hainline, Ph.D.
University of California

Cooperating Units: Department of Anthropology, University of California, Riverside, California

Man Years

Total	:	0.08
Professional	:	0.08
Other	:	None

Project Description:

Objectives: To provide an additional criterion in our effort to identify the genetic interrelationship among the peoples of Micronesia and Western Pacific in general.

Methods Employed: The study involved the evaluation of the fingerprints collected from 444 individuals from the District of Yap during a recent anthropological study.

Major Findings: The results indicated that: 1) this group has one of the highest incidences of whorls reported anywhere, 2) arches were of very low frequency, 3) there were no statistically significant sex differences in the distributions of the fingerprint patterns, and 4) there were no significant differences between the left and right hands of either sex.

Significance to Biomedical Research and the Program of the Institute: This project contributed to the further understanding of the genetic composition of this population.

Proposed Course of Project: None. Study completed.
Honors and Awards: None Publications: None

Serial No. HD-CD14(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Five-Year Registry Study of ALS/PD Cases on Guam

Previous Serial Number: NDB (CF) - 62E 961

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: Dwayne M. Reed, M.D.
University of California
Teresita S. Elizan, M.D.
Perinatal Research Branch, National Institute of Neurological Diseases and Blindness
Leonard T. Kurland, M.D.
Mayo Clinic

Cooperating Units: Department of Epidemiology, School of Public Health, University of California, Berkeley, California
Section on Infectious Diseases, Perinatal Research Branch, National Institute of Neurological Diseases and Blindness
Section of Medical Statistics, Mayo Clinic, Rochester, Minnesota

Man Years

Total	:	0.10
Professional	:	0.10
Other	:	None

Project Description:

Objectives: The role of heredity in the etiology of ALS has been a debatable issue since the discovery of the disease. Guam, with it peculiarly high prevalence of ALS, presents an excellent testing ground for the genetic hypothesis. In 1958, a point survey of patients and controls were conducted by Doctor Robert Krooth, a geneticist and former member of the Perinatal Research Branch. Even though the results of that survey strongly suggested that ALS occurred more often among the sibs of patients than one would expect by chance alone, the data failed to show the same trend among the offspring and the parents of patients. A similar analysis of the PD cases failed to offer conclusive indications of familiality mainly because of the

limited amount of data available at that time. In view of the inadequacy of the point survey, a new prospective study was initiated in 1958 by Doctor Krooth, with the following objectives: (1) to determine whether a familial concentration of the disease exists in both ALS and PD; (2) to determine if there is a significant tendency for a parent-offspring aggregation in these diseases; and (3) to conduct general comparisons among the relatives of the patients and controls.

Methods Employed: The basic principle of the prospective study was the accumulative registering of each and every new patient and a suitable control during a five year period, and if necessary, during a ten year period, after the initiation of the study. The living parents, sibs, offspring, and spouses of both patients and controls were likewise registered. Complete family histories were also recorded.

Case finding among the relatives of patients and controls consists of a thorough neurological examination; any suspicious or doubtful "cases" are reexamined by the staff neurologists, and either confirmed or rejected as a case.

Major Findings: Because of the possibility that ALS and PD are two forms of the same disease spectrum, the data for ALS and PD were pooled and analyzed as if we were dealing with one disease, ALS/PD.

After five years of existence the patient-control panels were closed and over 80 percent of 2,000 relatives registered were located and given complete neurological examinations. These examinations have been completed. The main results of this study are:

(1) ALS/PD of Guam is familial. At lease 23 percent of the registered patients showed definite history of the disease in one or more of their 0.5 relatives (parents, sibs or offspring). The familiality value of 23 percent is considered a minimal one since only relatives affected during the years 1953-1964 were considered and then only if clinical and/or pathological records of diagnosis were available in our NINDB files.

(2) The brothers of the registered ALS/PD patients have a higher incidence of the disease than the general male population of Guam or the brothers of the registered controls (weighted by age and sex). The sisters of the patients also demonstrated a tendency for higher incidence; however, statistical comparisons gave nonsignificant differences.

(3) The degree of parent to offspring transmission (a main indicator of genetic transmission) could not be estimated at this stage. This was due to the fact that the ages of the parents and offspring were well above and below the ages of high risk for ALS (respectively) which resulted in a time. It is hoped that an answer may be obtained within five to ten years when most of the offspring of both patients and controls will advance to the ages of high risk.

Significance to Biomedical Research and the Program of the Institute: To determine the degree of familial occurrence of this disease and to provide evidence for the probable role of heredity in its etiology.

Proposed Course: This project has been completed.

Honors and Awards: None

Publications: Plato, C.C., Reed, D.M., Elizan, T.E., and Kurland, L.T. "Amyotrophic lateral sclerosis/parkinsonism dementia complex of Guam. Part IV. Familial and genetic investigations." Amer. J. Hum. Genet. 19:617-632, 1967.

Serial No. HD-CD15(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Pedigree Studies in Umatac

Previous Serial Number: NDB (CF) - 63 E 1090

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: Manuel T. Cruz
National Institute of Neurological Diseases and Blindness Research Center
Leonard T. Kurland, M.D.
Mayo Clinic

Cooperating Units: National Institute of Neurological Diseases and Blindness Research Center, Agana, Guam
Section of Medical Statistics, Mayo Clinic, Rochester, Minnesota

Man Years

Total	:	0.15
Professional	:	0.15
Other	:	None

Project Description:

Objective: Umatac is a small village in southern Guam which has the highest rate of ALS/PD on the island.

Several pedigrees collected earlier from this village suggest the possibility that ALS/PD is a genetically controlled disease transmitted through a single dominant gene with incomplete penetrance. This has not been confirmed since in a village as small and isolated as this one, with a presumably high degree of inbreeding, chance alone might be responsible for the high prevalence of familial occurrences.

The objectives of the present study are: (1) to trace and construct pedigrees for all the ALS/PD patients in the village; (2) to estimate the degree of consanguinity in this village; and (3) to evaluate the role of inheritance regarding the high prevalence of ALS/PD in this village, and to establish a possible mode of genetic transmission through segregation analysis.

Methods Employed: The first phase of this project has been completed. Almost 97 percent of the village population was formed into a single pedigree which we were able to trace back for nine generations. Preliminary inbreeding coefficients of all 232 sibships were calculated. Coefficients of relationships between sibships with one or more ALS/PD patients and sibships with none affected were also calculated.

Major Findings: (1) The mean inbreeding coefficient of the sibships with one or more ALS/PD patients was very close to that of the sibships without affected individuals. This finding tends to argue against a recessive type of inheritance. (2) Sibships with one or more ALS/PD sibs are more closely related to each other than the general population. This is suggestive of dominant inheritance. (3) The segregation analysis of the various types of sibships offers evidence that ALS/PD as found on Guam is probably an inherited disease transmitted through a dominant gene which, if corrected for age, is completely penetrant in the males, but about 50% penetrant in the females. A manuscript has been prepared and submitted for publication to the American Journal of Human Genetics.

Significance to Biomedical Research and the Program of the Institute: The investigation of the pedigrees of this village will assist in defining possible genetic factors involved in the etiology of ALS/PD. If a recessive factor is responsible, a higher inbreeding coefficient must be observed in the sibships with one or more affected than those with no affected individuals. On the other hand, if dominance with incomplete penetrance is responsible, then a higher coefficient of relationship will result between affected vs. affected sibships, than the other comparisons that affected vs. normal or normal vs. normal sibships.

Proposed Course of Project: This project has been completed.

Honors and Awards: None

Publications: None

Serial No. HD-CD16(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Possible Intrauterine Interaction Between Twins Incompatible for the Xg^a Blood Group

Previous Serial Number: NDB (CF) - 67 E 1492

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: Joseph D. Mann, M.D.
Butterworth Hospital
T. Theodore Schwartz, M.D, Head
Epidemiology Branch, National Institute of Neurological Diseases and Blindness

Cooperating Units: Butterworth Hospital, Grand Rapids, Michigan
Epidemiology Branch, National Institute of Neurological Diseases and Blindness

Man Years

Total	:	0.15
Professional	:	0.05
Other	:	0.10

Project Description:

Objectives: The purpose of this study is to determine the possibility of intrauterine interaction between dizygotic twins incompatible for the Xg^a (sex linked inherited) blood group; and if such interaction does take place we then propose to estimate its effect upon the monozygotic:dizygotic twin ratio.

Methods Employed: Blood samples are to be drawn from approximately 500 pairs of twins who are participating in the ophthalmologic investigations to be conducted by the Epidemiology Branch (NDB (CF) - 66 E 1254). The Xg^a blood type determinations are to be carried out by the laboratories of the Butterworth Hospital.

Major Findings: Project still in progress.

Significance to Biomedical Research and the Program of the Institute: To investigate the possible intrauterine interaction and loss of dizygotic twins due to the Xg^a blood group incompatibility.

Proposed Course of Project: The project is continuing. Three hundred pairs of twins (mostly monozygotic) have been studied. The collection of the data continues with the majority of the remaining twin pairs being dizygotic.

Honors and Awards: None

Publications: None

Serial No. HD-CD17(c)

1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Serologic and Dermatoglyphic Evaluation of a Group of Mentally Retarded Children

Previous Serial Number: None

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: None

Cooperating Units: None

Man Years

Total	0.10
Professional	0.05
Other	0.05

Project Description:

Objectives: This represents a routine study of the blood groups and dermatoglyphics of the patients studied at the Children's Diagnostic and Study Branch.

Methods Employed: Blood specimens are collected in heparinized tubes and the blood groupings are performed through the use of commercial reagents for the following systems: ABO (A_1, A_2, B, Ax), Rh (C, $\bar{c}$, D, E, $\bar{C}$, C^w, D^u), Duffy, Kell (K, $\bar{K}$, Kp^9, Kp^6), Kidd, Lewis (Le^a, Le^b), Lutheran, MNS (M, N, Mg, S, $\bar{s}$, U), R and P.

Major Findings: Study is still in its initial stages.

Significance to Biomedical Research and the Program of the Institute: It is a further effort to establish possible associations or genetic linkage between known genetic markers (in this case blood groups and dermatoglyphics) and the various types of mental deficiency.

Proposed Course of Project: To continue the collection and accumulation of data.

Honors and Awards: None Publications: None

Serial No. HD-CD18(c)

1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Blood Groups, Haptoglobins and Dermatoglyphic Patterns of the Patients with ALS-PD Complex of Guam

Previous Serial Number: NDB (CF) - 67 E 1491

Principal Investigators: Chris C. Plato, M.S., Geneticist
Ntinos Myrianthopoulos, Ph.D.
Perinatal Research Branch, National Institute of Neurological Diseases and Blindness

Other Investigators: None

Cooperating Units: Perinatal Research Branch, National Institute of Neurological Diseases and Blindness

Man Years

Total	:	0.05
Professional	:	0.05
Other	:	None

Project Description:

Objectives: The object of this study is to estimate the frequencies of the various blood group, haptoglobin and dermatoglyphic patterns among the ALS-PD patients and compare them with those of the normal Chamorro population in an effort to determine possible association between ALS-PD and known genetic markers.

Methods Employed: Ninety ALS-PD patients were ascertained and blood typed for the ABO, Rh, MNS, Kell, Duffy, P, and Diego systems. Haptoglobin and hemoglobin determinations were carried out by starch gel electrophoresis. Presently we are engaged in the analysis of the finger and palm prints.

Major Findings: The completed results of the blood group comparison between the ALS-PD patients and the normal Chamorros showed significant differences in respect to two systems. (1) The patients possess lower frequencies of the P_2 gene; and (2) The frequency of Diego positive among patients is significantly higher than that of the normal Chamorros. Studies on the fingerprint comparisons are still in progress.

Significance to Biomedical Research and the Program of the Institute: To study the distribution of ALS-PD in an effort to find possible associations of this disease with already established genetic markers.

Proposed Course of Project: Blood group work has been completed. Need to evaluate the dermatoglyphic data and interpret the results.

Honors and Awards: None

Publications: None

Serial No. HD-CD19(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Individual Case Study: Behavior of an Infant with Congenital Anesthesia

Previous Serial Number: None

Principal Investigator: Judith L. Rapoport, M.D., Psychiatric Consultant

Other Investigators: None

Cooperating Units: None

Man Years

Total	:	0.1
Professional	:	0.1
Other	:	None

Project Description:

Objectives: Developmental Psychology and Child Psychiatry have been concerned with the relation of somatic stimuli to early attachment behavior. There exists considerable debate between those emphasizing physiological satisfaction and secondary drive reduction as the basis for early object attachment and those stressing the adequacy of social stimuli. The case study of a child with marked sensory defects has relevance for this debate.

Methods Employed: Mother and child will be seen on a monthly basis both to provide counseling for the mother and to collect behavioral data. Observations on the strength of attachment, intellectual development, and the quality of mothering behavior will be made in some detail. Behavior will be filmed at regular intervals and at least one home visit will be made.

Major Findings: Findings to date have been apparently normal emotional development, with a full range of affective expression, age-appropriate attachment to mother, and stranger anxiety. The data thus far are supportive of those concepts of emotional development which stress the importance of maternal responsivity rather than the fulfillment of specific physical need, or specific sensory stimulation.

Significance to Biomedical Research and the Program of the Institute: The basis for attachment behavior is relevant to normal development and to clinical entities (such as infantile autism) in which emotional attachment has failed to develop normally.

Proposed Course of Project: Continued observations are planned over approximately a one year period. If intellectual development is adequate, futher cognitive testing may be indicated.

Honors and Awards: None

Publications: None

Serial No. HD-CD20(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Maternal-Infant Interaction in High-Risk Mother Population

Previous Serial Number: None

Principal Investigator: Judith L. Rapoport, M.D., Psychiatric Consultant

Other Investigators: Joan C. Mahle, M.S.W., Clinical Social Worker
Alice Abramson, M.A., Education Specialist
Felix de la Cruz, M.D., M.P.H., Chief

Cooperating Units: None

Man Years

Total	:	0.24
Professional:		0.1 (1 person)
		0.1 (1 person)
		0.02 (1 person)
		0.02 (1 person)
Other	:	None

Project Description:

Objectives: This program has two principal aims: (1) to study the effect of stress during pregnancy on maternal attitudes toward the child and on mother-infant interaction during early infancy, and (2) to study the effect of parity on maternal reaction to stress and on mother-infant interaction.

Methods Employed: A variety of rating techniques are planned for studying the mothers during pregnancy, the neonate, and mother-infant rearing practices. The clinical population will be composed of women attending the National Naval Medical Center Obstetrical Clinic. Twenty women designated as medically high-risk and an equal number of controls will be selected during the last trimester of pregnancy. Each group will consist of 10 primigravida and 10 with one normal child.

During the last month of pregnancy, the sample of forty mothers will be given a taped psychiatric interview and a standardized pregnancy research questionnaire. These will be rated for physical symptoms of discomfort during

pregnancy, depression, fantasy of infant defect, acceptance of female role, marital happiness, attitudes toward maternal role, and specific fantasies and attitudes toward the particular pregnancy in light of its having some risk for the child.

The infants will be examined during the first four days of life by a pediatrician on the staff of Children's Diagnostic and Study Branch, who will conduct a neonatal neurological examination in order to assess the infant state of responsivity.

Observations of the maternal-infant interaction according to the standardized procedures of Moss will be collected during two home observations: at age one month and age three months.

Major Findings: The project is in its initial stages of development. Data collection will begin during July, 1968.

Significance to Biomedical Research and the Program of the Institute: An important factor in the development of a handicapped child is the maternal attitude toward defect. We anticipate that the high risk group will be made up of mothers who have a high incidence of fear and stress during pregnancy but who are statistically likely to have normal offspring. We expect to find that stress alone, in the absence of neonatal pathology, will produce differences in mother-infant interaction between the high risk and the normal groups.

Proposed Course of Project: After an inter-rater reliability is established for mother-infant interaction, full scale data collection will begin. In future research this data may serve as control data for studying a third group of mothers whose infants have defects diagnosed at birth.

Honors and Awards: None

Publications: None

Serial No. HD-CD21(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Comparative Study of Chromosome Preparations

Previous Serial Number: None

Principal Investigator: Fredrica Santell, B.S., Biological Laboratory Technician

Other Investigators: None

Cooperating Unit: None

Man Years

Total	:	.06
Professional	:	.06
Other	:	None

Project Description:

Objectives: The plan was to produce better metaphase spreads that were consistently well-spread and well-defined, which would permit a more precise karyotype analysis.

Methods Employed: Short term tissue cultures of peripheral blood from humans were carried out using a modification of Jackson's micro-technique. Two different types of phytohemagglutinin (Difco and Burroughs Welcome) and three types of hypotonic solutions (distilled water, 0.5% sodium citrate, and 0.4% potassium chloride) were used.

Major Findings: This study revealed that hypotonic potassium chloride solution gave a higher percentage of metaphase spreads that were consistently well-spread, well-defined, and without overlap, than other hypotonic solutions used. Also, in a comparison of two different brands of phytohemagglutinin, both were found to be comparable in mitogenic activity.

Significance to Biomedical Research and the Program of the Institute:
Better preparations permit a more accurate microscopic observation in studies of chromosomes.

Proposed Course of Project: Continue to improve the chromosome preparations, employing similar designs.

Honors and Awards: None

Publications: This work appeared as an abstract in the Mammalian Chromosomes Newsletter 9: 1, 43, January 1968.

Serial No. HD-MR20(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Changes in Parental Expectations About Their Retarded Child: Effects due to parental variables and diagnostic counseling

Previous Serial Number: Same

Principal Investigator: Adam P. Matheny, Jr., Ph.D., Department of Pediatrics, University of Louisville

Other Investigator: Joel Vernick, M.S.W., Clinical Social Worker

Cooperating Unit: University of Louisville School of Medicine, Department of Pediatrics, Louisville, Kentucky

Man Years

Total	:	0.09
Professional:		0.01 (1 person)
		0.08 (1 person)
Other	:	None

Project Description:

Objectives: This program consisted of two interlocked studies: 1) Discrepancy Formation by Parents About Their Mentally Retarded Child, and 2) Parental Counseling Assessment: Relation to Changes in Parental Expectations for Their Retarded Child.

The studies have been broadly concerned with: a) the accuracy with which parents view their retarded child's present behavior, b) the relation between the parents' view of present behavior and future expectations, and c) how both the view of present behavior and future expectations are affected by diagnostic counseling.

Methods Employed: The first study has established parental estimates and expectations regarding their child's abilities, problem areas, educational level, etc. Forty families have been studied extensively before and after their children were seen at the Children's Diagnostic and Study Branch. Relations studied centered upon: 1) socio-economic variables, 2) age, sex, and parity number of the child, 3) type of handicap diagnosed, 4) severity of

the handicap, 5) sex and age of the parent, 6) social abilities of the child, and 7) parent-child interaction.

The second study has focused upon diagnostic counseling itself. Records kept on several pediatricians' counseling sessions with parents show relations among: 1) the dynamic exchange of information, 2) the invariant modes by which information is exchanged, and 3) the "success" with which a counselor's style engenders changes in parental behavior.

Major Findings: These studies indicated that an experience with a diagnostic clinic almost invariably changes parental views of their child in the direction of more realism. Also it is found that the physicians who counsel parents promote different degrees of change with differing degrees of success because of their own counseling strategies.

Specific relations of variables mentioned above have been analyzed and a paper, "Parents of the Mentally Retarded Child: Emotionally Overwhelmed or Informationally Deprived?" is being prepared for publication.

The data have already been gathered with regard to the effectiveness of various styles of parental counseling in a Clinic concerned with mental retardation. This material will be included in a forthcoming paper.

In addition to the original sample of 40 we have added ten more cases. This increase of cases was utilized in order to carry out further statistical analysis on the effect of sex and age of the child diagnosed upon parents receiving clinical information.

Significance to Biomedical Research and the Program of the Institute: Despite the prevailing view that parents of the handicapped child have intrinsic psychological problems which deter the effective progress of the child, it also seems likely that the handicapped child subverts the normal patterns of a well-functioning family. To establish the variables involved in disordered family management is important for counseling and for suggesting management practices to parents of the retarded child.

Proposed Course of Project: In the near future it is expected that this program can be employed on families with infants "at risk" for developmental anomalies. Family expectations, family coping patterns and degrees and types of professional involvement can be related to family changes over time. Data have been collected and the project will be terminated this year.

Honors and Awards: None

Publications: None

Serial No. HD-CD22(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: An Investigation of Aspects of Eye Separation Distance on the Human Face as a Phenotypic Trait

Previous Serial Number: None

Principal Investigators: Waldemar H. Wenner, M.D., Medical Officer
Ronald P. Bachman, M.D., Clinical Associate

Other Investigators: None

Cooperating Units: None

Man Years

Total	:	0.1
Professional	:	0.1
Other	:	None

Project Description:

Objectives: 1) To develop a means of obtaining reliable measures of certain facial characteristics. 2) To develop criteria related to these facial characteristics which would allow them to be explored as to the validity of their use in the delineation of medical syndromes. 3) The use of these criteria in the evaluation of syndromes that are commonly considered to have unusual facial characteristics to determine if such unusual facies can be objectively evaluated. Specific plans now call for the study of hypertelorism.

Methods Employed: It was decided to obtain facial photographs and so far the greatest effort has been the designing of an apparatus to assist in this. The assistance of Mr. W. Stotler of the Biomedical Engineering and Instrumentation Branch of the Division of Research Services and others of that Branch has allowed this to progress to the point where we now have the apparatus and we can begin using it in pilot studies.

The apparatus is constructed to hold the subjects' head (a modified surgical head holder is used) in a fixed distance from a camera and so that a line through the external auditory canals (a modified radiographic, cephalometric, head holder is used) is perpendicular to the optic axis of the camera. As

designed, the subject is placed horizontally looking up into a mirror angled 45° from the vertical and interposed between him and the horizontally directed camera which is fixed ten feet from a line through the external auditory canals. In addition, mirrors at both sides of the head allow lateral views of facial characteristics as well. The equipment is designed to be portable.

Measurements will be taken directly as well as from photographs.

Major Findings: Project is in its initial stages of development.

Significance to Biomedical Research and the Program of the Institute: One area of significance is the exploration of a method to objectify and systematize the description of the human face with present efforts being directed toward the spacing of the eyes and the position of the ears as specific components of adequate facial description. The human face is thrust into a position that justifies this attention because certain variations in it have extremely widespread use as medical diagnostic criteria despite there being inadequate objectification of the variability that can be expected. This study, in addition to working out a method of investigation, also hopes to gather normative data with regard to facial development.

Proposed Course of Project: After using the apparatus to evaluate its capabilities, it will be used to obtain photographs from a group of children such as those at the Hospital for Sick Children, Washington, D.C. as a means of determining the sample size necessary for a normative developmental study of facial characteristics. In addition, specific study of populations of individuals who demonstrate various syndromes with unusual facial characteristics will be studied by these means.

Honors and Awards: None

Publications: None

Serial No. HD-CD23(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies of Newborn Behavior

Previous Serial Number: None

Principal Investigators: Waldemar H. Wenner, M.D., Medical Officer
Thomas C. Douthitt, Ph.D.
Child Research Branch, National Institute of Mental Health

Other Investigators: Mary E. Burke
Patricia A. Keenan

Cooperating Units: Newborn Nursery, National Naval Medical Center
Child Research Branch, National Institute of Mental Health

Man Years:

Total	:	1.8
Professional	:	1.8
Other	:	None

Project Description:

Objectives: 1) To evaluate for interjudge reliability an observational scheme of acquiring information about infant behavior over relatively long time spans. This has been accomplished and a report is being prepared for publication detailing the methods and reliabilities obtained. 2) To use the above scheme in classifying observable behavior into sleep and waking states having neurophysiologic concomitants known from other investigators' work. This was done to again allow assessment of the interjudge reliability of such a classification. 3) To explore the sequence characteristics of infant behavior; this involves several approaches: a) to explore the sequences of the immediately observable behavior, b) to explore the sequence of sleep and waking states infered from the immediately observable behavior, and c) to explore the periodicity of certain behaviors in relation to a Basic Rest-Activity Cycle postulated by other workers. In this part of the study it is hypothesized that sequence behavior may not only be individually differentiating at a very young age, but also may reflect the status of the

central nervous system with regard to maturity, functional ability and possible previous trauma. 4) To study the maturation of the premature infant's behavior through longitudinal follow-up during nursery stay using the above-mentioned observational scheme. 5) To assess the short-term stability of behaviors studied with the above-mentioned observational scheme.

Methods Employed: Notations are made by judges every ten seconds when an electronically generated click is presented at earphones. The notational code used is a modification of Parmelee (Biol. Neonat., 4: 317, 1962). Generally, observations are of several hours duration. Also, almost always, the observational period of one day is repeated the following day so the stability of findings can be assessed.

Major Findings: 1) A previously undescribed periodicity of rhythmic oral movement groups has been found. It is present in the youngest prematures studied and has a period in infants of 30 to 60 minutes. 2) The observational scheme and the translation of these behaviors into sleep-wake states both show acceptable interjudge reliability.

The former finding has been presented as a paper at the Association for the Psychophysiological Study of Sleep meeting in the Spring of 1968 and is in press for publication--see below. The latter findings are being prepared for publication in a review of the literature concerning observational criteria of infant behavior.

Significance to Biomedical Research and the Program of the Institute: The attempt to "get a handle on" the, often considered chaotic, behavior of newborns through study of sequences offers promise on at least two fronts if it is successful. It would enable one to enlarge available diagnostic criteria for this age. Secondly, it might form another portion of the base from which longitudinal developmental studies of the human can be launched. Specifically, the exciting finding that one can cull a periodic phenomenon from such data, suggests the possibility of using this type of behavioral organization for diagnostic and research efforts.

The finding of acceptable interjudge reliabilities concerning infant behavior using observational criteria over long periods, which has not been adequately assessed by others, makes potentially available a useful methodological tool to scientists in this area.

Proposed Course of Project: a) Except for the rhythmic mouthing periodicity, the specification of behaviors stable over days has not been accomplished. The search will continue. Stability has been looked for only in a very preliminary way through the comparison of Transition Matrixes generated from a postulated Markov Chain model applied to the data, but it is felt this avenue holds promise. b) It is hoped that variables affecting the rhythmic mouthing periodicity can be further specified through study of additional infants. c) Microanalysis of the individual rhythmic mouthing bursts through use of visual recording techniques of T.V. recording or movies is being actively considered. d) Attempts to instrument the rhythmic mouthing response are reported as successful by others and it is hoped that use of these to

make long term, e.g. 24 hour records of this behavior will be possible. This would allow one to relate the emergence of the circadian periodicity to the subcircadian cycle we have found. e) The comparison of a stabilimeter with our observational scheme is now underway in an additional effort to ease the heavy burden of continuous personal observation of behavior.

Honors and Awards: None

Publications: Wenner, W. H., Douthitt, T. C., Burke, M. E., and Keenan, P. A.: Observations on the regular recurrence of groups of spontaneous rhythmic oral activity in infants. Presented at The Second Symposium on Oral Sensation and Perception, sponsored by the National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland. James Bosma, M.D. (Ed.). Springfield, Illinois, Charles C. Thomas (In Press).

ANNUAL REPORT OF THE GERONTOLOGY RESEARCH CENTER

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

JULY 1, 1967 - JUNE 30, 1968

Fiscal year 1968 was marked by the completion of the Gerontology Research Building at the Baltimore City Hospitals. Occupancy of the new building was taken during the period February to May 1968. In addition to moving laboratories of the Gerontology Research Center, programs of guest scientists from non-government agencies were assigned space in the building after appropriate review of their proposed research programs. Collaborative studies between investigators of the Gerontology Research Center and investigators of the following institutions are now in progress: University of Maryland Medical School; the Baltimore City Hospitals; Johns Hopkins University School of Hygiene; Pennsylvania State College; the Fels Research Institute; Sinai Hospital (Baltimore); Harvard University School of Public Health; Psychology Lab, Cambridge University (Great Britain); Johns Hopkins University School of Medicine; University of Pennsylvania; V. A. Hospital, Bay Pines, Florida; University of Michigan School of Medicine, Ann Arbor; McCollom-Pratt Institute, Johns Hopkins University; Institute for Experimental Gerontology, Basel, Switzerland, and V. A. Hospital, Baltimore, Maryland. Thus procedures have been established to develop the Gerontology Research Center as a regional resource for expanding research in gerontology.

The new Gerontology Research Center was formally dedicated on June 15, 1968.

During the year an Instrumentation Section has been established which has been of great value to investigators in all sections of the Gerontology Research Center. For example, a problem-solving computer has been designed and constructed. This instrument has greatly facilitated the administration of tests of problem-solving ability in the Section on Cognitive Behavior. A P-wave detector has also been designed and constructed which permits the investigator to present a stimulus to the subject at any specified time within the cardiac cycle. Other standard instruments in the laboratory have been modified to increase their reliability and to extend their capability to meet special experimental needs.

The goals of the Gerontology Research Center are to describe in quantitative terms the biochemical, physiological, and behavioral changes that take place with aging and to determine the basic biological mechanisms of these changes with the ultimate aim of reducing the impairments and disabilities of older people.

Attainment of the first goal involves a detailed study of age changes in normal men. These studies are based on a series of biochemical, physiological and psychological tests which are made at 18-month intervals on the

same individual. This is a long-term longitudinal study of approximately 650 community residing males who range in age from 18 to 97 years. The tests are administered within a number of different sections of the Center, but the entire program is centrally coordinated through the maintenance of all test results for ultimate computer analysis.

The second goal of the Gerontology Research Center, viz., determination of the basic biological mechanisms of aging, involves studies of aging in a wide variety of animal species. In addition, it is often necessary to investigate the details of cellular functions before specific studies of age differences can be fruitfully pursued.

Current biological theories of aging may be classifed as genetic or non-genetic in nature. Genetic theories of aging are based on the presumption that the life span of a cell (or animal) is pre-determined in the genetic code. Individual differences in life span within a species with uniform genetic composition are assumed to result from variations in environmental conditions. Hence, many studies conducted by the Gerontology Research Center are attempting to define the extent to which a given genetic program can be influenced by environmental factors.

Another biological theory of aging assumes that errors of transcription from the genetic code account for impairments in cellular function which impair performance and ultimately result in cell death. Numerous studies conducted by investigators in the Gerontology Research Center are attempting to test such hypotheses of aging.

Non-genetic biological theories of aging assume that with the passage of time key molecules undergo structural changes which alter their properties and interfere with their normal functions in cellular processes. The formation of cross-linkages in complex protein molecules is regarded as an important characteristic of aging. Such theories are being tested, especially with respect to collagen and elastin.

In addition to these cellular theories, the hypothesis that aging is due to a breakdown of control mechanisms, at both the cellular and total animal level, is being explored.

Although progress has been made in specific facets of all of these theories, it is not yet possible to present a unified theory of aging, or to exclude any of these theories from further consideration. Much work remains to be done.

This report summarizes the current status of research in the Gerontology Research Center under two major headings: (1) Physiological and Behavioral Aspects of Aging, and (2) Cellular Mechanisms of Aging.

Highlights of findings from the Gerontology Research Center include the following:

1. In a problem-solving task the presentation of redundant information increases errors in the elderly, whereas it does not influence the

performance of the young. Interspersing positive and negative information in the presentation to subjects did not lead to more errors in young than in old subjects. Thus the old were not more "rigid" in their thought patterns than the young.

2. Maze learning in old animals was significantly improved when trials were increased in each practice period. For example, mazes, which were readily learned by young animals, could not be learned by old animals under the same training regime. However, the old animals did learn the maze when massed trials were given at each testing. Thus it is possible that learning deficits in the elderly may be substantially reduced by altering teaching methods from those which have proved most effective for children and young adults.

3. The age difference in the blood glucose response to tolbutamide cannot be ascribed to differences in blood level of tolbutamide.

4. The increasing reliance of clinical investigators on the "rapid" intravenous glucose tolerance test has led to a critical evaluation of factors influencing the logarithmic decay in plasma glucose concentration with time. The decay constant, k, is distinctly higher in arterial than in antecubital venous plasma. Furthermore, k varies significantly from vein to vein. Glucose concentrations in artery and peripheral veins are, however, nearly identical 60 minutes after glucose injection. This single point then is probably a more reliable indicator of glucose tolerance than the cumbrous k value--if venous samples must be used.

5. The glucose utilization of human leucocytes in vitro is the same in young and old subjects. Leucocytes from diabetic patients use more glucose than those from non-diabetic subjects; this enhanced utilization is true of insulin-requiring diabetics, of diabetics controlled by sulfonylurea therapy, and even of "chemical" diabetics. Glucose uptake was not increased by either exogenous or endogenous insulin.

6. An increase in lysosomes or lysosome-like structures has been observed in aging hydranths of Campanularia flexuosa and in late generation chick fibroblasts cultured in vitro. These findings lend support to the hypothesis that lipofuscin age pigment is the result of changes which occur in these particulates with time.

7. In rats, a long lived RNA (greater than 30 days, compared with 1-10 days for known forms of RNA) has been found in brain and skeletal muscle. A long lived RNA may undergo changes with time which induce errors in protein formation which impairs cellular function and ultimately results in cell death.

8. The rate of turnover of mitochondria has been determined in brain, liver, heart muscle, and kidney in old and young rats. Half-lives of H^3-leucine labeled mitochondria were found to vary from 8.4 days (liver) to 24 days (brain), but no significant differences were found between 12 and 24-month-old rats. These results do not support the "error" hypothesis of

aging, which would predict a slower rate of turnover in mitochondria in old than in young animals.

9. The accumulation of lipofuscin age pigment in the adrenal cortex of the rat is accelerated in animals maintained on a vitamin E deficient diet. These results suggest that a deficiency in this known lipid antioxidant may accelerate age pigment formation.

10. There was a 50% decrease in the rate of incorporation of 2-14 C acetate into fatty acids in the livers of senescent rats. In contrast, incorporation of the tagged acetate into liver glycogen increased 3-4 fold in the senescent rats.

11. The characteristics of parthenogenesis, poikilothermia, and a short life span make the rotifer an ideal organism to study the effects of environment on genetically programmed aging. Methods have been developed to culture rotifers in a defined medium which will permit quantitative estimates of food intake. Life span of rotifers has been increased 3-4 fold by feeding 1/1000 of the standard number of bacteria. This increment is considerably greater than the 33% increment previously observed by restricting the diet by feeding algae and fresh pond water at 2-3 day intervals.

12. Evidence for a novel mechanism for the active transport of glucose in the kidney and intestinal mucosa involving the enzyme trehalase, which hydrolyzes the disaccharide trehalose to two glucose moieties, has been obtained. The presence of trehalose has been demonstrated in kidney and intestine in eleven different animal species. Enzyme systems for the synthesis of trehalose have also been identified in these tissues.

13. There is a significant decline in respiratory control of oxidative phosphorylation in mitochondria from brains of 27-month-old rats compared with those from 11-month-old animals.

14. Detailed measurements have shown that collagen fibers aggregate by both surface reactions and between polypeptide chains at a molecular level but the two reactions proceed independently.

15. Segments of the DNA molecule can be blocked and unblocked by the binding or removal of histones. The degree to which DNA is blocked influences the informational transfer required for protein synthesis and the greater binding of histones to DNA in old than young organisms has been offered as an explanation of biological aging. It has been shown that metal ions are of crucial importance in the binding of histones to DNA. Metal ions compete with DNA for histones primarily by attachment to the arginines. Thus the regulation of information transfer by DNA through histones is in turn regulated by metal ions. Two histone components that are separable only in the presence of divalent metals in young animals cannot be separated even in the presence of those metal ions in old animals. Previous suggestions that the nature of the DNA histone binding changes with age can thus be interpreted at least in part as resulting from age differences in the metal ion environment of the nucleohistone.

16. It has been found that zinc ions induce a specific cleavage of uracil phosphodiester bonds which occurs on both sides of the uracil (at the 3' and 5' positions). A cyclic phosphate intermediate has been discovered. It has also been found that zinc ions will degrade only RNA and not DNA. Thus treatment with zinc offers an extremely useful method for separating DNA from RNA from complex mixtures.

17. It has been shown how both activation and inhibition can occur with the same metal ions (at different concentrations) acting on the same enzyme. At lower concentrations the metal ions bind to histidine groups on the activating site, whereas at higher concentrations they bind to other histidine groups. This latter effect changes the three-dimensional structure of the enzyme so that it is no longer capable of interacting with substrate.

PHYSIOLOGICAL AND BEHAVIORAL ASPECTS OF AGING

The prospective study of human aging continues to be characterized by the intensiveness and diversity of the studies performed on each visit (made possible by the willingness of the subjects to spend 2-1/2 days at the Baltimore City Hospitals) and by the good health and loyalty of the study group (only 5% have died and almost 90% of those living remain active participants over approximately 10 years).

Of critical importance for the analysis of aging trends in specific functions for individual participants is the fact that of the 708 participants almost 400 of them have been examined at least three times, 281 four times, and 166 five times. Accurate identification of individual aging patterns are difficult because of relatively high intrinsic measurement variance associated with relatively low mean rates of change with age.

Methodology in the area of analysis of prospectively-developed multiple data points is receiving some preliminary attention. Thus 47 subjects over age 70 have had four or more visits. A variety of variables were examined with an eye to detection first of distinct age changes in individuals and secondly of the pattern of change when present. For illustration, the data on vital capacity (VC) reveals important trends. Thirty-seven of the 47 subjects showed a decrease in VC, and only one increased. The even more interesting point is that in a number of those who showed a decline in function there appeared to be two distinct levels of performance; that is, the decline was not a relentless downhill process, but a plateau which dropped between discrete visits to a new lower steady-state level. Other subjects, however, did show the smoothly declining pattern. Thus possibilities which previously were discussed only in theoretical terms have now been demonstrated to occur in actuality. The validity and significance of these results will depend upon correlation with decremental patterns of other variables in the same subjects and with careful and unbiased review of clinical information available on each subject on each visit.

Another interesting development in methodology seeks to take advantage of the father-son (F-S) pairs in the study in order to evaluate the role of genetic factors in aging. F-S pairs were compared with pairs of unrelated subjects who matched the F-S pairs with respect to age, height, and smoking habits. No genetic impact on pulmonary function could be demonstrated by this technique. Alternative analytical techniques which do not require matching are being developed.

The prospective nature of the Longitudinal Study has made it possible to demonstrate that deleterious effects of cigarette smoking on pulmonary function may be ameliorated when smoking is discontinued. Comparisons of function were carried out in 10 subjects (with appropriate controls) before and after they stopped smoking. Specifically, a marked and consistent reduction of mixing ratio and therefore presumably greater uniformity of distribution of air in the lungs occurred.

An exercise test has been standardized which promises to be highly suitable for the determination of physical fitness, especially in older subjects. A bicycle ergometer has been modified so that it can be turned by supine subjects with their arms. The amount of exercise is less than the maximum work rate that they could attain, but greater than the work rate they could maintain under steady-state conditions for long periods of time. This intermediate level of work was one-half the maximal rate achieved during a brief (15 second) period of exercise. Thus the advantages are: (1) arm exercise avoids the problems of leg infirmities very common in older people, (2) recumbency permits reliable comparison of cardiovascular responses before, during, and after exercise, and (3) exercise rate is determined by the individual's own physical capacity and is therefore predictably moderate.

It is relatively simple to demonstrate that the performance of older people is poorer than that of a younger group in tasks of learning and problem solving. The important study of the mechanisms underlying these decrements is the continuing interest of the Section on Cognitive Behavior. Better delineation of factors which improve performance--and of factors which worsen performance--is needed both for its theoretical importance and for possible practical applications to problems of learning and memory in aging individuals. Thus the evidence is now strong that the addition of a second modality (hearing) to a variety of visual learning tasks results in significant improvement in performance. Under conditions in which an interfering set of stimuli is interposed between the initial learning task and the later test set, auditory participation during learning improved performance in both young and old, but more so in the old. Since the improvement provided by active auditory participation (the subject read the information aloud) was only slightly better than that provided by passive auditory participation (the experimenter read the information aloud) it was not clear whether the critical factor was simply the addition of a second sensory modality or whether active participation by the subject might provide significantly more benefit. Further experiments will be needed to answer this. In one set of studies it was shown that the provision of an interference set of stimuli is not critical for the demonstration of this auditory improvement; in a learning task without interference, subjects showed improved performance with auditory input and this was particularly true of the old subjects. In another set of studies a geometric form had to be reproduced; again auditory input (the experimenter described the form aloud) resulted in substantial gain for an older group of subjects.

The decreasing ability to reason, that is, to synthesize learned information in order to solve specific problems continues to be studied in

older people. Information needed to solve problems may be presented either in positive form (what the concept is) or in negative form (what the concept is not). Furthermore, certain discrete bits of information may be redundant in the sense that they repeat a fact which was previously presented. Provision of information by these varying techniques and noting their effects on the efficiency of problem-solving could provide clues in pin-pointing the step or steps in reasoning that deteriorate with age. Thus, perhaps contrary to expectations, interspersal of information presented in positive form with information presented in negative form did not lead to more errors for old than for young. In this sense then the old were not more "rigid" in their thought patterns than the young--they were as able to handle the changing character of the information as the young. In the second set of studies, however, the addition of redundant (unneeded) information resulted in more errors for old than for young subjects, who indeed did not commit errors secondary to redundancy.

The animal behavior program of the Section on Physiological and Animal Psychology has been analyzing the effects of aging on such psychological processes as drive and motivation, learning and memory, and sensation and perception. Underlying these studies is the long-term objective of identifying those mechanisms which are responsible for deleterious age changes so that techniques can be discovered for ameliorating those changes.

One interesting approach to the development of experimental models for hypothesis-testing has resulted in the finding that certain behavioral differences in genetically discrete mouse strains resemble the age differences that occur within a single strain. Thus, the exploratory activity of old mice is lower than that of young mice. A similar difference in activity which is, however, not age-dependent, is also present in two strains of mice of the same age. Factors which may be found to modify behavior in the inactive strain so that it resembles behavior in the active strain then could be tested to see whether it will be effective in delaying or reversing changes that occur with increasing age.

One finding which was reported last year is that old animals tend to show behavioral rigidity, and that this rigidity tends to interfere with learning, and even with taste discrimination. Research this year has indicated that this effect can be overcome and that problems which have been previously reported to be insoluble by aged rats can be learned by them if they are properly trained. Specifically, we observed that many old rats who were trained in a 14-unit T maze were unable to learn the maze when the training trials were distributed (i.e., four trials per day). However, when the training trials were massed (i.e., 12 trials per day) these non-learners were able to learn. This was true even though the total number of trials was equal in the two groups. Furthermore, during the distributed training conditions, we observed that the errors were not random throughout the maze, but tended to occur at the same cul-de-sac for a given animal--but not the same for each animal. Thus the failure to learn was attributable to a tendency among these old rats to perseverate their errors. This conclusion is further supported by the results from extinction trials. The senescent animals continued to run to the goal box longer than did young animals

even though the food was no longer available.

A new program in the Section on Physiological and Animal Psychology has been activated this year. The two scientific themes being developed are (a) an analysis of the interactions between cardiovascular regulation and behavioral regulation, and (b) an analysis of the extent to which, and means by which, cardiovascular systems can be brought under voluntary control.

In the past year the major progress has been in the establishment of the technical aspects for several different laboratory studies. Three of the projects are studies in man. (1) Liaison with the Cardiovascular Section of the Department of Medicine at the Baltimore City Hospitals has been established so that patients with certain cardiac arrhythmias can be identified for subsequent hospitalization and study on the Gerontology Research Ward. Attempts will be made to teach these patients to control their heart rates and rhythms by techniques previously shown to be effective. (2) The interrelations between reaction time, cardiac cycle phase, and respiratory cycle phase will be studied by improved techniques in which the stimuli for the reaction time study can be introduced during pre-chosen segments of the cardiac cycle. The technique is based upon computer analysis of time-relations in cardiac and respiratory cycles preceding the cycle during which the test stimulus is to be given. (3) Psychometric and intellectual evaluation of patients before and after introduction of cardiac pacemakers will test the hypothesis that improvement too subtle to be detected by usual clinical evaluation may result from this new therapeutic procedure.

The Endocrinology Section has developed a radiochemical (double isotope derivative) method for the measurement of peptides and especially for the hormonal peptide, angiotensin. This material is involved in the pathophysiology of several varieties of hypertensive disease, and its measurement, and that of the enzyme renin which produces it, is required for diagnostic, and hence therapeutic, purposes. Research in this area has been limited by dependence on bioassay techniques. As a consequence, we and others have attempted to provide more generally useful methods for the measurement of these important materials. The radiochemical method which has been developed here will be applied to measurements of human plasma after an adequate supply of human hormone has been made available by synthesis, an effort which is currently in progress in the Endocrinology Section. Availability of human angiotensin (decapeptide) will also permit us to undertake the development of immunoassay methods for this peptide.

Other activities in the Endocrinology Section have dealt with several interrelated studies: the age related alteration of thyroid hormone metabolism in man, the determinants of plasma free (non-protein bound) thyroxine levels during infectious disease, and gel electrophoresis techniques for studying thyroid hormone binding by plasma proteins. In the past year these studies have led to a re-evaluation of the role of thyroxine-binding prealbumin (TBPA) as a determinant of acute changes in plasma free thyroxine. This protein, contrary to current views, has been shown to be only a minor carrier of thyroxine in human plasma. While changes in TBPA concentration do occur acutely, the changes do not condition the acute changes

of free thyroxine one sees in a variety of illnesses. Further studies into the nature of these protein-hormone interactions are underway.

There is now increasing recognition of the role of aging as a confusing variable in interpretation of diagnostic tests for diabetes. The subjects of the Longitudinal Study Group continue to provide a valuable resource to the Metabolism Section for its investigation of the mechanism of these age decrements in metabolic performance. Realistic standards for interpretation of four clinically-used diagnostic tests for diabetes are being derived in a computer-based study now being programmed at Bethesda. Tests conducted in the past year have now provided us with a large series of subjects who have had all four diagnostic tests and an even larger number who have had three of the four different tests performed. Statistical techniques have been developed which will permit comparison of age-corrected performance on the four tests among the subjects. Such questions as relative specificity and sensitivity of the tests can now be examined and overall accuracy can be judged by analyzing correlations of performance on the individual tests with other clinical and biochemical variables known to be characteristic of the overt diabetic state.

In one of the tests used, the intravenous tolbutamide response test, the effect of age on the tolbutamide concentration curve in plasma has been studied. A surprising variation in biologic half-time was found--ranging from 3.5 to 22.5 hours in 26 subjects--but age did not influence the disposal rate of tolbutamide. Older subjects did show a significantly higher concentration of tolbutamide 6 minutes after the rapid injection was made than the young. This finding is contrary to the expectation that the test dose (given per unit of body weight) would lead to initial concentrations of tolbutamide which would be the same in all age groups or might be lower in the older than in younger subjects. This intriguing finding suggests age differences in binding of tolbutamide to plasma albumin but evidence for this remains to be obtained.

A further significant advance in methodology for the double-antibody immuno-assay for insulin has been developed. Adaptation of AutoAnalyzer modules has successfully automated the two phases of the assay.

The intravenous glucose tolerance test has been gaining favor among clinical investigators in the past 2 or 3 years. The reason for this is probably that the multiple glucose concentrations obtained during the study can be expressed in terms of a single parameter, the half-time, or the "k" value (percent fall per minute). Examination of some of the previously unexplored variables which influence the k value have now shown that the chance selection of one of the antecubital or forearm veins may have a significant effect on interpretation of the results, since venous k values are usually lower (never higher) than arterial k values. In contrast, the 60-minute glucose concentration is nearly identical in the arterial and in all venous samples. This finding suggests that a single venous sample one hour after infusion may be a more reliable index of glucose tolerance than a k value derived from multiple venous samples.

Leukocyte metabolism was examined as a possible prototype for in vitro studies on carbohydrate metabolism of "isolated tissue" from human subjects. Glucose uptake (Q_G) by leukocytes was measured. Metabolism of the cells was remarkably stable. Age of the donor had no influence on Q_G nor did exogenous insulin (added to the incubation flask), nor did endogenous insulin (cells obtained one hour after glucose ingestion). Three types of diabetics, however, had significantly greater glucose uptake than did the non-diabetic group. Leukocytes from "chemical" diabetics, overt diabetics on sulfonylurea therapy, and diabetics requiring insulin did not differ from each other, however.

CELLULAR MECHANISMS OF AGING

Modern developments in biology have emphasized the importance of understanding the mechanisms of cellular processes in order to provide rational approaches to dealing with many of the diseases which play a role in impairments in the elderly, such as arthritis, vascular disease, diabetes, etc. Previous studies from the Gerontology Research Center have indicated the importance of the gradual loss of cells from many key tissues, such as muscles, nerves, kidneys, which takes place with aging and contributes to a gradual loss of reserve capacities in many organ systems. Hence a part of the resources of the Center have been devoted to studies of cellular biochemistry and the structural characteristics of molecules involved in biological processes.

The Section on Cellular and Comparative Physiology has continued ultrastructural studies of the very short-lived marine colonial hydrazoan, Campanularia flexuosa, with the view of locating the subcellular components subject to structural changes during the rapid aging of this organism. In common with cells of mammalian tissues it was found that in all but two of the cell types of the hydranths there was a notable increase with age in primary and secondary lysosomes and residual bodies (all organelles thought to be associated with intracellular digestion). This result is in agreement with earlier studies indicating an increase in lysosomal enzymes in aging hydranths. No evidence is at present available to help decide whether this increase is due to an increased synthesis of lysosomes or to inability of the cell to degrade or excrete them.

An increase in lysosomal structures was also the most prominent aging change observed in another ultrastructural study comparing early and late generation chick fibroblasts cultured in vitro.

"Death" for the individual Campanularia hydranth involves cellular disorganization and efficient translocation of cellular material back into the remainder of the colony. That this process is markedly different from the usual form of "unprogrammed" death in other organisms was emphasized in a preliminary ultrastructural study of the regressing hydranth. At the onset of regression there is in almost all cells a generalized sequestering of mitochondria and other cytoplasmic material into giant autophagic vacuoles (2°

lysosomes) where degradation occurs. Recognizable cytoplasmic structures become almost entirely replaced by membrane-bound residual bodies containing osmiophilic material. Up to this point, at least, the cell nuclei remain remarkably unchanged in appearance, indicating that autolysis has been confined to the autophagic vacuoles. The process resembles in some respects the "intracellular autophagy" seen in starving cells of Euglena, although vastly greater in rate and magnitude.

The Section has continued studies of turnover of cell components in rodent tissues in a search for aging changes in the ability of cells to replace structural components and for the identification of structures and molecular species in addition to DNA and collagen which have low rates of replacement. Age changes in such long-lived elements might play a role in senescence since they are least likely to be corrected by molecular replication. Mice labeled early in life with C^{14}-leucine and sacrificed up to 160 days later did exhibit long-lived protein components, especially in particulate fractions of brain and skeletal muscle, and in the histones of most tissues. In another experiment involving mice injected with C^{14}-uridine the residual activity found in pooled samples of RNA from several tissues suggested the existence of a minor RNA component of relatively long half-life (greater than 30 days, compared with 1-10 days for known forms of RNA). More work will be required to identify the particular protein and RNA species involved.

An age comparison of the turnover of mitochondria in the rat has been extended to five other tissues besides the liver. Half-lives of H^3-leucine labeled mitochondria were found to vary from 8.4 days (liver) to 24 days (brain). In agreement with the earlier work of Fletcher and Sanadi with liver mitochondria, exponential radioactive decay was observed and no significant differences in half-lives were noted between age groups. Thus, in contrast to the whole organism, the "mortality rate" of mitochondria does not appear to vary either with the age of the mitochondrion itself or the organism as a whole.

Preliminary data have been obtained indicating that there are no substantial age differences in the rate of degradation of ribosomal RNA in brain, liver, kidney, lung, and spleen of the rat. The maximum uptake of the orotic acid label into the brain ribosomes of old rats was, however, considerably diminished compared to the young rat, suggesting a possible age effect on the blood-brain barrier.

Quantitative studies of intracellular lipofuscin pigment accumulation, begun a number of years ago in this laboratory with a study of the human myocardium, has been extended in detail to tissues of the Wistar rat. In contrast to the myocardium, certain other tissues of the aging rat, including Purkinje cells of the cerebellum, zona reticularis of the adrenal, testicular interstitial cells, and ovarian macrophages, were found to accumulate pigment volumes far greater than those seen in the senescent human myocardium. The fact that two of these cell types are producers of ketosteroid hormones may indicate a connection between ketosteroid metabolism and pigment formation.

The widely-held belief that lipofuscin age pigment contains products of lipid autoxidation received some support from a study of the distribution of lipofuscin-like pigment resulting from vitamin E deficiency in the rat. It was found that the pattern of pigment accumulation among the various cell types of the adrenal closely resembles that of the normal age pigment accumulation. The results suggest that the absence of this known lipid antioxidant may accelerate normal age pigment formation.

During the past year studies in the Section on Nutritional Biochemistry have been continued to test the concept that age differences are most marked in enzymes concerned with highly specialized functions of differentiated cells. In these studies glutamic acid decarboxylase activity was determined in the prosencephalon, brain stem, and cerebellum of 2-, 12-, and 26-month-old rats. The end product of this enzyme reaction is gamma-amino butyric acid (GABA) which plays an important role in brain metabolism since it is believed to (1) inhibit transmission of nerve impulses and (2) produce a source of energy by its further metabolism to succinic semi aldehyde and ultimately to succinic acid. This latter metabolic pathway may provide as much as 50% of the energy in brain tissue in certain physiologic conditions. However, no age differences in the enzymatic activity were observed so that the data do not provide support for the general concept.

One of the current hypotheses proposes that aging results from the oxidation of key molecules in the body by free radicals. Therefore, further studies have been carried out to determine the effect of age on the sulfhydryl content of plasma albumin. On the basis of estimates on 165 men, statistically significant age regressions ($P < 0.001$) in the sulfhydryl in plasma albumin was observed. However, the decrement over the age span 20-90 years was only 10%. Decrement of a similar magnitude was observed during the last 17 months of life in rats. Although small but statistically significant differences have been obtained to support this concept, their physiological importance must still be determined.

Renal clearances have been carried out in unanesthetized rats aged 12, 24, and 28 months. The results showed no differences between 12 and 24 month animals with respect to the inulin clearance or PAH_{Tm}. However, these renal functions decreased 33% in the 28-month-old animals. The uniformity of loss in these two functions suggests, as in the case of man, dysfunction or loss of individual tubules.

Further studies on the effect of age on metabolism of 2-14 C acetate by liver has been carried out. Unlike earlier studies carried out in the ionization chamber, a glucose load was administered prior to the injection of the radioactive isotope in order to stimulate certain metabolic pathways, so that age differences might be more apparent. Results showed that the incorporation of the isotope into fatty acids decreased 50% whereas that into glycogen increased 3-4 fold in the livers of 24-month-old as compared to 12-month-old animals. A small but not significant increase in incorporation into proteins was observed. However, other studies carried out in the section employing shorter experimental periods showed statistically significant increments in the incorporation of the isotope into proteins among old animals.

Further studies are necessary to identify the specific enzymes responsible for these striking age differences and to determine the fundamental causative factors.

During the past year efforts have been made to establish the mortality and fecundity in the rotifer colony since the culturing techniques have been revised to include the feeding of bacteria and defined media rather than algae and pond water. The results obtained on approximately 800 rotifers indicate a mean life span of 22.6 ± 0.20 days and an egg production of 52.2 ± 0.21 eggs per rotifer. Furthermore, the life span has been increased 3 to 4 fold by feeding 1/1000 the standard number of bacteria. This increment is considerably greater than the 33% increment previously reported due to dietary restriction when algae and fresh pond water were used as a source of nutrition. In addition, we have been unable to demonstrate an effect of parental age on the life span as reported in the earlier gerontological literature even after 50 generations.

The Intermediary Metabolism Section has initiated studies on regulation, modulation and control of biochemical systems and processes which show functional changes concomitant with senescence. These regulatory mechanisms have been examined, utilizing fresh and meaningful approaches, in selected aging systems, each possessing distinct advantages for experimental inquiry. New and significant information and concepts on the qualitative and quantitative changes in structure and function of organisms have been discovered and these will be of special value in determining and evaluating the basic origins and hypothesis of biological aging.

Highlights of findings from the Intermediary Metabolism Section include the following:

1. A novel mechanism for the active transport of glucose in the kidney and intestinal mucosa has been proposed. The enzyme trehalase, which hydrolyzes the disaccharide trehalose to two glucose moieties has been found in kidneys of eleven mammalian species, including man. The enzyme is specifically localized in the renal cortical tubules, the site of glucose resorption. The enzyme is membrane bound and cosediments with alkaline phosphatase, hexokinase, and 5'-nucleotidase activities. Trehalase is specific for trehalose. The enzyme has been purified 50 fold; the Km is 3.5 mM and the pH optimum is 6.3. Also found in the kidney cortex and intestinal mucosa are the enzymes that convert glucose to trehalose, namely, hexokinase, phosphoglucomutase, UDPG pyrophosphorylase, trehalose-6-P synthetase, and trehalose-6-P phosphatase. The enzymes have sufficient activity to account for the rate of resorption of glucose in the kidney of the intact animal. Phlorhizin, the classical inhibitor of sugar transport, inhibits the synthesis of trehalose by blocking formation of UDPG.

2. There is a significant decline in respiratory control (regulation by demand) of oxidative phosphorylation in mitochondria from brains of 27-month-old rats, compared with those from 11-month-old animals. This decline was observed with two substrates, succinate and α-ketoglutarate, but not with pyruvate or glutamate. Serum albumin improved the respiratory control with the mitochondria from old animals, suggesting that these

organelles are more susceptible to changes in environment. There were no age-related changes in the respiratory rate or efficiency of oxidative phosphorylation (P/O ratio) between the two groups.

3. Thyroid hormone was found to induce the specific synthesis of the mitochondrial α-glycerophosphate dehydrogenase. The activity in heart and liver for euthyroid, hyperthyroid, and hypothyroid rats are, respectively, 13.6, 41.9 and 2.7, and 7.9, 83.1 and 2.0 μmoles dihydroxyacetone phosphate formed x min^{-1} x g^{-1} mitochondrial protein. Perfused hearts from euthyroid rats have a rate of lactate utilization half that of pyruvate utilization. Because the lower rate of lactate oxidation may be due to a limitation in the availability of oxidized nicotinamide adenine dinucleotide (NAD, DPN), thyroid hormone was used to increase the flux of the α-glycerophosphate cycle, which, in turn, may increase NAD turnover. In a test of this hypothesis, it was found that the rate of lactate utilization by hearts of hyperthyroid rats increased 2-fold and equalled the rate of pyruvate utilization.

4. To determine the mechanisms of the decrease in work performance during aging in insects, regulation at the glycogen site was studied. Phosphorylase a, b and phosphorylase b kinase were found. The enzymes were purified and kinetic measurements on the cooperating effects of substrates, glycogen and inorganic phosphate, the activator, AMP, and the inhibitor, ATP, were ascertained to provide the necessary information for a determination of the control mechanism in vivo.

The Biophysics Section has continued to investigate the physical properties of aging connective tissue fibers. Previous years of work on several aspects of fiber cohesion was consolidated this past year by thoroughly measuring statistical variations of testing methods. Final data were obtained from the aging Wistar rats maintained at the Gerontology Research Center. The macroscopic size of tendon fibers, expressed as the ratio of weight/length (mg./cm.), increased rapidly between 1-3 months of age; however, fiber size increased only slightly thereafter between ages of 3-24 months. Mean values of tendon size were related linearly to body weight between 1-24 months. The distribution of fiber size was ascertained by sorting the number of fibers out of a total of 20 per tail into ten compartments 0.100 mg./cm. wide covering a total expanse of 0 to 1.00 mg./cm. Mean values computed for the number of fibers/compartment/6 tails showed a non-random distribution with respect to size. The overall increase in mean size observed with aging was delineated into a series of reactions that reflect serial lateral accretion of collagen. The peak number of fibers in 4-week-old tendons occurs at 0.100 mg./cm.; subsequent growth during 6, 8, and 10 weeks shifts the peak to 0.200 mg./cm. with progressive elevation of number in the 0.300 mg./cm. compartment. The peak then shifts to 0.300 mg./cm. at 12 weeks and plateaus out to 0.400 mg./cm. as age increases from 3 to 24 months. Re-evaluation of these findings on the basis of age as the independent parameter shows that percentage of fibers within a size exhibits a serial process of growth; viz., the number of small fibers reduces with age as the number of large ones increases. The latter never attains the high percentage of the small fibers that were present initially at 4 weeks of age. These findings show that collagen fibers grow rapidly by accretion

of soluble precursors (tropocollagen), or they are transformed into large fibers by a dynamic exchange reaction.

Inter-chain cohesion of collagen fibers at the molecular level also was measured by recording the rate of flow of melted tendon fibers under a standardized mechanical stress. The time required for a melted fiber to rupture was ascertained on 20 samples per tail, at ages between 3 and 24 months using 6 rats per age. Time-to-break increased linearly with increasing body weight of rats during rapid growth (up to 3 months of age); near termination of growth, time-to-break increased by a first-order rate process in excess of the extrapolated linear rate process. The exponential increase occurs, therefore, when tendon size attains a nearly steady-state size and size-distribution with respect to age. These measurements point out that collagen aggregates by surface reactions in fibers as well as between polypeptide chains at the molecular level. The two reactions are independent of each other since they follow a different course with respect to age.

Elastic stiffness is a third measurement of fiber cohesion which was observed to be a complex function of tendon size. Measurements made during this past year provide final statistical evaluation of how elastic stiffness depends upon fiber size and age. Data show that large fibers have lower elastic stiffness than small fibers when measurements are expressed as force/cross sectional mass and relative length. The dependence of stiffness on fiber size itself changes most rapidly during early growth, but little change occurs thereafter between 3 to 24 months. It also was observed that yield and tensile rupture stress increase with increasing fiber size. Thus, the decrease in stiffness of large fibers is compensated by increasing strength. Further investigation of this system is planned; particular attention will be given to the role of fiber orientation in age changes of tendon. Tensile properties of rat tail dermis follow a linear pattern with regard to age and body weight. Yield and rupture strengths and the modulus of elasticity increase with age and body weight. Most rapid advances occurred during early growth (1 to 3 months), with slow further increases between 3 and 24 months of age. These tests confirm some earlier work reported in the literature, but the most important result is that the tests were done with rings of skin dissected from rat tails. This measurement therefore has the potential value of being used to test living rats on a serial basis. The Biophysics Section therefore plans to determine whether or not it is possible to dissect rings of skin from rat tails serially in time while maintaining live animals on a long term study program. If this can be done and corrections made for accumulated wound response, then one can determine longitudinal effects of aging on cohesion and tensile properties of connective tissue in skin. This will be applied to the possibility of detecting life span of individual young animals on the premise that velocity changes in cohesion of collagen and elastin are involved in determining longevity.

Studies on the mechanism of hydration of aging connective tissue were continued by measuring the rate of dehydration of freshly dissected tissue (dorsal skin). Desorption is a complex time-dependent process, and the mechanism has not been fully evaluated. However, it was established that velocity of desorption changes linearly with quantity of water desorbed

in the tissue; data for 3 to 24 month old samples were significantly different from combined data of 1 month old tissue. A marked change occurs in the mechanism of water binding at 1 month, but the same mechanism appears to hold for ages between 3 and 24 months. The most outstanding changes with age were a decrease in water content and an increase in half-life for desorption occurring between 1 and 3 months of age. These findings show that rate of desorption is not constant but that it changes with age.

The endocrine regulation of tendon cohesion was studied by measuring changes in fiber size (mg./cm.) and time-to-break. Velocity of body weight gain (growth rate) abruptly declines when pituitary is removed. Rats hypophysectomized at 6 weeks of age continue to grow, but when hypophysectomy is done at increasing ages growth rate approaches that of controls. There is no difference in growth rate between experimental and control animals at an extrapolated age of 9 to 12 months. However, it is significant to point out that body weight of the experimental animal did not decline at this age. Cohesion of tendon fibers changes in proportion to response of growth rate to hypophysectomy. Mean tendon size is lower in hypophysectomized rats of 6 weeks of age than in controls; time-to-break, on the other hand, is higher in these hypophysectomized rats than in controls. The response of tendon two months following hypophysectomy at ages up to nine months is insignificant. This short term reaction of young rats to hypophysectomy confirms previous work reported in the literature. Long-term response (6 to 9 months) following hypophysectomy shows that fibers weaken and become disengaged. Specifically, the time-to-break is lower by 50% compared with controls. It is apparent that connective tissue responds to loss of pituitary function by at least two reactions, viz., a (2 month) fast depletion of loosely held fibers (reduction in size) then a slow (6 to 9 month) disaggregation of collagen chains (reduction in time-to-break). This transitory reaction may be parallel with cessation of pituitary control of growth rate in control animals that approach their growth plateau.

A new program of research started which emphasizes elastic fibers, elastolytic enzymes, and elastolytic enzyme-inhibitors. Work to date has concentrated on developing methods for investigating rat tissues since previous studies utilized human, calf, and rabbit organs. These sources provided needed quantities of tissue in which elastolytic enzymes could be detected readily. Since the emphasis in current plans is on age changes in these enzymes, it is necessary to work with rats with known life histories. Some advance has been made in adapting the enzymatic methods to tissues obtained from rats. The new program on elastin and elastolytic enzymes and the continuing program on collagen fibers complement each other. Both deal with effects of aging on structural and metabolic regulation of cohesion in connective tissue of important physiological organs.

One of the important steps in the copying of the genetic information, both in the replication of the genetic material DNA and in the transcription of the RNA messenger which sends the information to the site of protein synthesis in the cell, is the unwinding of the double-stranded DNA helix. After replication and transcription the DNA must be rewound into the double helical form. The Section on Molecular Biology has now discovered that divalent

metal ions are generally capable of bringing about both unwinding and rewinding of DNA. The metals vary in their unwinding effectiveness, those binding "bases" most strongly being the best catalysts for this process. They vary even more greatly in their ability to regenerate the double helix by forming crosslinks between the separated DNA chains. Quantitatively, some metals can bring about total regeneration whereas others cause only a small amount of the duplex to reform. Qualitatively, the metals that produce total regeneration do so under a variety of conditions of temperature, ionic strength, etc., that depend on the relative affinity of the metals for "base" and phosphate sites. It is therefore obvious that the nature and quantity of metal ions present during the genetic information transfer processes can play a vital role either in their stimulation or repression.

The degradation of RNA by zinc ions is of importance as a tool in the study of the nucleotide sequence that determines the genetic information. We have discovered that specific cleavage of uracil phosphodiester bonds, which makes the reaction useful, occurs on both sides of the uracil (at the 3' and 5' positions). We have uncovered a cyclic phosphate intermediate, indicating that this zinc degradation proceeds in some respects like the degradation of ribonuclease enzymes which are responsible for the removal of RNA when the latter has served its biological function. We have demonstrated that zinc ions will degrade only RNA, and not DNA. This finding is extremely useful for the removal of RNA from DNA, and has already been applied in other laboratories. RNA and DNA are frequently found together in the cell; our method for their separation (when the recovery of only DNA is desired) is the mildest technique that has been developed.

The effect of metal ions on enzymes, the proteinaceous catalysts of virtually every biochemical reaction, has been little understood, since metal ions are able to either activate or inhibit such enzymes. In the case of the ribonuclease enzyme (mentioned above) we have been able to show how both activation and inhibition can occur with the same metal ions acting on the same enzyme. At low metal concentration the metal ions apparently bind to histidine groups on the activating site, whereas at higher concentration they bind to other histidine groups; this latter effect changes the three-dimensional structure of the enzyme so that it is no longer capable of interacting with substrate. This appears to be the first instance in which the different metal effects on an enzyme have been capable of rational interpretation.

Among the foremost biological problems under consideration at this time is the question of how parts of the genetic information of DNA are unused during one portion of the life of an organism and then become available during another portion; this problem is the basic problem of cellular differentiation. Evidence has been accumulated indicating segments of the DNA molecule can be blocked and unblocked by the binding or removal of proteins called histones. We have demonstrated that metal ions are of crucial importance in the binding of histones to DNA. In the presence of divalent metal ions four histone components can be obtained from a DNA-histone complex from rat liver. In the absence of these metals only two of the components are produced. The histone fractions that are most susceptible

to the action of metals are those with a high content of arginine, indicating that metal ions compete with DNA for histones primarily by attachment to the arginines. These findings suggest that the regulation of DNA by histone is in turn regulated by metal ions. It is of great interest, therefore, that it is possible to correlate age-dependent histone fractionations with metal-ion dependent fractionations. The same two histone components that are not obtainable in the absence of divalent metals in "young" animals are also not obtainable even in the presence of metal ions in "old" animals. Previous suggestions that the nature of DNA histone binding changes with age can thus be interpreted at least in part as resulting from age changes in the metal ion environment of the nucleohistone.

Serial No. HD-AG3(c)
1. Gerontology Research Center
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Longitudinal Studies of Human Physiology, Biochemistry and Psychology.

Previous Serial Number: HD-AG3 (c)

Principal Investigators:
N. W. Shock, Director (15% time)
R. Andres, Clinical Director (15% time)
A. H. Norris, Coordinator (40% time)
P. Davis (15% time)
M. Epstein (15% time)
M. Haas (15% time)
A. Johnson (15% time)
W. Reichel (15% time)
L. Shenkman (15% time)
M. Weisfeldt (15% time)
T. Weiss (15% time)

Other Investigators:
D. Arenberg
C. H. Barrows
R. I. Gregerman
C. Martin
J. Avruch
J. Gregory
D. Shreiner
W. Weglicki

Cooperating Units:
Baltimore City Hospitals
Dr. Bernice H. Cohen
School of Hygiene and Public Health
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Baltimore, Maryland
Mrs. Marjorie Zukel
Heart Disease Control Program
U.S. Public Health Service
Washington, D.C.
Dr. Robert B. McGandy
Dept. of Nutrition
Harvard School of Public Health
Boston, Massachusetts

Dr. Benjamin Bell
Mr. Charles Rose
Boston V. A. Normative Aging Study
Veterans Administration Outpatient Clinic
Boston, Massachusetts
Dr. Richard Feinberg
National Institute of Neurological Diseases
and Blindness
Bethesda, Maryland

Man Years:

Total:	8.77
Professional:	1.90
Other:	6.87

Project Description:

Objectives: This project is designed to: (1) facilitate repeated measurements of various physiological, biochemical and psychological variables in the same individuals as they grow older, (2) coordinate the utilization of the results of these measurements in establishing indices of aging and (3) compare the effects of socio-economic status on these results and the indices which may be derived therefrom.

Methods Employed: Subjects are volunteers recruited by other participants. They agree to be available for a three-day series of tests every 18 months for the rest of their lives. A given series of tests will be repeated at a five year interval. Measurements of body size and composition, pulmonary function, kidney function, neuromuscular function and cardiovascular function are made. Standardized psychological tests include the Guilford-Zimmerman Temperament Survey, Southern California Tests of Creative-Thinking Abilities (Word Fluency, Associational Fluency I, Expressional Fluency, Ideational Fluency I, Consequences), Benton revised Visual Retention Tests and the vocabulary subtests of the Wechsler Adult Intelligence Scale. Also included are two verbal learning procedures of appropriate difficulty to provide a wide distribution of scores for the longitudinal subjects. Physical examinations and medical histories are performed with check lists to assure uniformity of recording. Medical findings and pertinent test results are reported to a participant's private physician only. Participants are treated as members of an elite group and kept informed of the progress of the total program and of test results for the group. Data are kept in restricted files and are summarized by calculating machine and electronic computer methods.

Major Findings: During the past year the longitudinal studies program has emphasized: 1. calculation, coding and editing of previously collected data which will be placed in the data storage and retrieval system, 2. development of an automated data storage and retrieval system for the program, and 3. continuation of pilot evaluation and summary of data which has been punched into tabulation cards and edited. Data is placed in the data storage system twice a year. For example, visit cards were edited and updated

through December 31, 1967. This has provided a distribution of participants by date of birth: before 1880 - 7; 1880 to 1889 - 58; 1890 to 1899 - 120; 1900 to 1909 - 133; 1910 to 1919 - 180; 1920 to 1929 - 151; 1930 to 1939 - 49; 1940 to 1949 - 10. Of this total group of 708 participants, 47 have withdrawn from the program, 37 have died and 29 are inactive for the present. This leaves 595 active participants. Deaths were distributed by date of birth as follows: before 1880 - 2; 1880 to 1889 - 14; 1890 to 1899 - 10; 1900 to 1909 - 8; 1910 to 1919 - 3. Repeat testing is now being performed on schedule. We have now tested 708 participants one time, 532 two times, 397 three times, 281 four times, 166 five times, 78 six times, 24 seven times, 5 eight times and 1 participant nine times.

Statistical methodology has been developed to assess the similarity of characteristics of pairs of individuals who are related as compared to pairs who are unrelated. Pulmonary function measurements (vital capacity, forced expiratory volume at 0.5 sec. and forced expiratory volume at 1.0 sec.) were chosen for the first comparison of 43 father-son pairs and 86 other participants who were matched to each father and each son for age, height and smoking status. Pulmonary function measurements for each individual (father, son, match) were compared with prediction equations based on pulmonary function, age, height and smoking status of 647 longitudinal studies participants. Each of these multiple regression computations was also performed for the group of participants under 50 years old and for the group 50 years of age and older.

The initial hypothesis tested was that the fathers and sons varied from their expected pulmonary function levels in the same direction. The expected pulmonary function levels were determined from the multiple regression models derived from the study population. If the difference between the observed and expected pulmonary functions carried the same sign for the father and the son (i.e. both positively differed from their expectations, both negatively differed from their expectations, or neither differed from his expectation) a pair was considered concordant in the direction of its deviation and coded as positive. Discordant results were coded a negative. Significance of the distribution of positive and negative results was examined with a sign test.

Matching by age height, and smoking habits eliminated the three variables which would most easily account for a difference between the two groups. To examine the hypothesis that the difference in pulmonary function between fathers and sons was less than that for their matches, a t-test was performed. The t-test treated each pair difference as a unit of observation.

A t-test was also performed on the differences in pulmonary function for fathers and their matches versus sons and their matches to investigate the possibility that the prediction equations were not appropriate for the sub-samples of matched pairs. After substituting for the pulmonary function of the match an expectation calculated from the multiple regression equations, the appropriateness of the prediction equations was again exa-

mined with the t-test but without the problem of random variation introduced by the use of the matched individuals. While the hypothesis was not supported, the data showed a high degree of internal consistency.

Results of measurements which are performed at each visit have been examined for age changes in participants over 70 years of age who have had four or more visits for testing. There were 29 such participants who were 70 to 74 years of age, 14 who were 75 to 79 years old and 4 who were 80 years of age or older. Measurements of hemoglobin, cholesterol, glycerides and creatinine in the blood were examined as were 24-hour creatinine clearance and excretion values. Systolic and diastolic blood pressure, basal metabolic rate, height, weight, skinfold thickness and pulmonary function measurements were also examined. Age trends were identified in the vital capacity and 24-hour creatinine excretion results. Of 29 participants in the 70-74 year age group 21 showed a decrease in vital capacity while 8 showed no change. Of the 21 who showed a decrease 7 showed a decrease from a higher to a lower plateau of function each of which was maintained for two or more visits. Of 14 participants in the 75-79 year age group 13 showed a decrease in vital capacity while one showed an increase. In the 80+ year age group three of four participants showed a decrease in vital capacity and one showed no change. While the changes with age for 24-hour creatinine excretion were clearly identifiable, the distribution of trends was in contrast to the vital capacity trends. In the 70-74 year age group 22 of 29 participants showed no change while 6 showed a decrease and one showed an increase in 24-hour creatinine excretion. In the 75-79 year age group 10 participants showed no change in function while 4 showed a decrease in 24-hour creatinine excretion. In the 80+ year age group 3 participants showed a decrease in 24-hour creatinine excretion and one showed no change. The step decrease from one plateau to another was found for three of the participants in the 70-74 year age group and one each in the two older age groups for 24-hour creatinine excretion. The identification of this step decrease in function in addition to the gradual decreases in function requires that our theoretical and analytical models of age change accept both patterns and provide for the examination of the relationship between the step and other events.

Proposed Course of Project: Collection of this body of data will place us in a position to test theories of aging against the human population of the longitudinal studies. Results of comparisons between old and young individuals are presently available, but these do not permit us to evaluate theories of aging adequately. Our ability to do so will depend in part upon a comparison of the integrated medical, physiological, psychological and biochemical profiles of the individual participant with various subsequent outcomes which we may be in a position to monitor in the same individual. Outcomes such as length of life, occurrence of disease, rate of change in a functional parameter, among other things, will be sought. Some theories assume that a few cells are lost during each unit of time so that in an individual or in an individual organ system changes of a gradual nature would be found. These theories are consistent with the summation of a series of distributed minor accidents (such as radiation hits) if only a few cells are involved each time. Other theories would

relate large changes in function with the occurrence of a disease episode, or major accident resulting in a substantial loss of cells. These theories are consistent with abrupt changes in functional levels which would be maintained until another major accident results in a new level. In addition, these two widely different types of theories could be found in combination with sudden as well as slow changes operating simultaneously. Rapid access to these complex relationships in individual participants will be invaluable in reaching conclusions about the appropriateness of theoretical formulations for this population.

Honors and Awards:

1. Dr. Shock was invited by the Soviet Academy of Medicine to participate in a conference on Symposium on Adaptive Capacities of an Aging Organism held in Kiev in June 1967.

2. Dr. Shock was invited by the Japanese Geriatric Society to give the keynote address at their annual meeting in Nagoya, Japan, October 1967.

3. Dr. Shock was invited to deliver a lecture on aging by the Age and Opportunity Bureau, Winnipeg, Canada in January 1968.

4. Dr. Shock was invited by the Swiss Academy of Medicine to participate in a seminar on Experiment Gerontology held in Basal, Switzerland in February 1968.

Publications:

Shock, N. W.: Current trends in research on the physiological aspects of aging. J. Amer. Geriat. Soc. 15: 995-1000, Nov. 1967.

Shock, N. W.: The physiology of aging. In Powers, J. H. (Ed.): Surgery of the Aged and Debilitated Patient. Philadelphia, W. B. Saunders Co., 1968, pp. 10-43.

Shock, N. W.: Current trends in biological research on aging. Jap. J. Geriat. 5: 21-27, Jan. 1968.

Serial No. HD-AG4(c)
1. Gerontology Research Center
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Age Changes in Human Performance

Previous Serial Number: HD-AG4 (c)

Principal Investigators: A. H. Norris (30% time)
Allen Johnson (85% time)

Other Investigator: N. W. Shock

Cooperating Units: Baltimore City Hospitals
Dr. A. T. Welford
The Psychological Laboratory
University of Cambridge
England
Dr. Robert W. Ramsey
Medical College of Virginia
Richmond, Virginia
Dr. Robert D. Teasdall
School of Medicine
The Johns Hopkins University
Baltimore, Maryland
Dr. John Magladery
School of Medicine
The Johns Hopkins University
Baltimore Maryland

Man Years:

Total:	3.79
Professional:	1.15
Other:	2.69

Project Description:

Objectives: This project is designed to study the effects of aging on: (a) the physiologic responses to exercise, (b) the rate of recovery of physiologic equilibrium after exercise, (c) muscular efficiency and (d) work output and fatigue. In addition, the factors responsible for limitations of performance observed in older people will be evaluated.

Methods Employed: Measured amounts of physical work are obtained in sub-

jects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake, CO_2 elimination, pulmonary ventilation volume, heart rate, blood pressure, and cardiac output (by the dye method) are taken before, during and after standardized amounts of exercise. In addition, the volume of the lungs and functional capacities of the pulmonary system are evaluated. Other studies include measurements of speed of nerve conduction, reflex delay time, and muscle action potentials. These phenomena are recorded on a six channel oscillograph or dual beam oscilloscope as the experiment demands.

Major Findings: The effects of cessation of smoking on pulmonary ventilation has been measured in 10 participants in the longitudinal studies of aging. Mixing ratio (the ratio of the actual to ideal number of breaths required to reach an alveolar nitrogen concentration of 2%) was used to measure pulmonary ventilation. Measurements were made before and after participants ceased smoking entirely. Mixing ratio was reduced in every case indicating that the lungs approached a more ideally ventilated volume. The smallest decrease in ration was 5 while the largest was 62 (average decrease = 41). The average mixing ratio for the ten participants during smoking was 178 compared with 137 after they had stopped smoking. It is concluded that the cessation of smoking results in more uniform pulmonary ventilation.

A standardized exercise test has been developed which permits the evaluation of both old and young men in terms of their physiological responses to an intermediate level of work (less than the maximum work rate they could attain and greater than the work rate they could continue until the responses reached equilibrium). The arm exercise task was not the same for all participants but was chosen to have a fixed relationship to the measured power generating capacity of their arms. This maximum power generating capacity was determined during a brief (15 second) period of cranking of a manually operated ergometer. Physiological responses (minute volume, oxygen uptake, carbon dioxide elimination, and heart rate) were measured during and after exercise at one-half the maximum work determined for each participant. The duration of exercise for all age groups was approximately 1.25 minutes. In this time the 30-39 year group performed the greatest amount of work at the highest rate (771 Kg. M. at 666 Kg.M./min.) while the 70-79 year group performed the smallest amount of work at the lowest rate (615 Kg.M. at 479 Kg.M./min.). The work rate performed by the oldest group was 72% of the work rate performed by the youngest (30-39 year) group. Similarly, ventilation volume, oxygen uptake and carbon dioxide elimination in the 70-79 year old participants were 75%, 70%, and 69% respectively of the responses measured in the 30-39 year old participants. Heart rate responses in the oldest participants were 90% of the heart rate responses in the youngest participants. These responses were similar in magnitude to responses to steady-state work reported in numerous earlier studies. Maximum levels of responses in the present study were reached in 0.1 to 0.3 minutes after the end of exercise or approximately 0.5 minutes after the end of the one-minute work rate plateau. Thus, for young and old participants alike it was possible to

obtain responses to submaximal work of short duration which were equivalent to responses to steady-state exercise performed at the same rate. Moreover, the responses reached maximum levels after the end of exercise. It was not possible, therefore, to attribute the limitation of participants' ability to continue the work rate to a limitation of any of the responses measured. It is likely that some factor in the local environment of the muscle or in the muscle itself was responsible for the decline in work rate.

Proposed Course of Project: Appraisal of participants in the longitudinal studies will be based on measurements of muscle strength and maximum power generating ability during arm exercise. Cardiovascular, ventilatory and metabolic responses to standardized arm ergometer exercise and monitered treadmill exercise will be used to classify participants into fitness categories. Measurements of lung volumes, uniformity of pulmonary ventilation, lung diffusing capacity, pulmonary compliance and thoracic gas volume will be made to characterize the respiratory competence of the longitudinal studies participants and to evaluate the benefits of the cessation of smoking in this group. The immediate results of cessation of smoking will be followed in staff members.

Honors and Awards:

1. Mr. Norris was invited to present a lecture at the Research Conference on Applied Work Physiology sponsored by the New York University Medical Center, Institute of Rehabilitation Medicine at Sterling Forest, New York in April 1968.

2. Mr. Norris was invited to participate in a symposium on Aging and Physical Activity sponsored by the American College of Sports Medicine at University Park, Pennsylvania in May 1968.

Publications:

Mittman, C.: Nonuniform pulmonary diffusing capacity measured by sequential CO uptake and washout. J. Appl. Physiol. 23: 131-138, July 1967.

Edelman, N. H., Mittman, C., Norris, A. H., and Shock, N. W.: Effects of respiratory pattern on age differences in ventilation uniformity. J. Appl. Physiol. 24: 49-53, Jan. 1968.

Serial No. HD-AG5(c)
1. Gerontology Research Center
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Age relationships of body composition, nutrition and physical activity.

Previous Serial Numbers: HD-AG5 (c) and HD-AG14 (c)

Principal Investigators: A. H. Norris (30% time)
R. Andres (15% time)

Other Investigators: N. W. Shock
C. H. Barrows, Jr.
R. I. Gregerman
M. J. Yiengst
C. Martin

Cooperating Units: Baltimore City Hospitals
Dr. Harold Schraer
Department of Biophysics
Pennsylvania State University
University Park, Pennsylvania
Dr. Stanley M. Garn
Fels Research Institute
Antioch College
Yellow Springs, Ohio

Man Years:

Total:	2.99
Professional:	0.45
Other:	2.54

Project Description:

Objectives: This project is designed to describe age differences in body composition, nutrition and physical activity made concurrently in individual subjects, to examine the relationship of these age differences, and to compare them to selected physiological and biochemical characteristics of the subjects.

Methods Employed: Height and weight are obtained by the usual anthropometric methods. The volume of the body is measured by its displacement of helium in a closed chamber. The density of the body is calculated

from these measures after correction for total skeletal mass estimated from skinfold thickness and roentgenographic techniques. Estimates of indices of lean body mass include: (1) basal metabolic rate determinations made with standard open circuit methods, (2) urinary excretion of creatinine and (3) body water and fluid distribution determinations made from the distribution curves of injected antipyrine and sodium thiocyanate. Nutrient intakes and eating habits are estimated from diet diaries and interviews. Physical activity levels are estimated from interviews and self-administered questionnaires. This is a continuing program. These studies are carried out on people of different ages and on the same people as they become older.

Major Findings: Tables of average weight for a broad height and age span have been constructed by adding the data on aged subjects collected by Master to the data from the National Health Survey. Participants in the longitudinal studies have mean relative weights by age decade groups which are 98.1% for the 30-39 year age group, 98.8% for the 40-49 year group, 98.3% for the 50-59 year group, 100.8% for the 60-69 year group, 102.5% for the 70-79 year group and 105.3% for the six participants over 80 years old when compared to the average values from the table. While there are few extremely fat men among the longitudinal studies participants, the hypothesis that fatter men are more jolly than thinner man was examined by comparing relative weight to the Friendliness scale on the Guilford-Zimmerman temperment survey. The highest correlation coefficients of -0.231 were found for the 40-49 year age group and 60-69 year age group. Not only were these correlation coefficients too small to be significant but their negative signs opposed the hypothesis. The correlation coefficient including all age groups was even smaller (<0.1) and negative in sign.

The possibility that participants who were gaining or losing weight would report low or high physical activities was examined. Gain or loss of weight was defined as a trend which included the visit before and the visit after the physical activity estimate (visits were separated by a year or more). Average values of reported physical activity were similar for weight gain, weight stable and weight loss groups. This result is attributed to the small absolute size of the weight trends and possibly to a lack of sensitivity of the activity report.

Proposed Course of Project: We hope to determine whether the lower mass of tissue in persons over 70 is: (1) an unfavorable sign associated with disease or a change in nutrition or activity or (2) a result of making measurements in a selected group, the aged survivors of a larger cohort. Individual patterns of bodily changes will be followed in the participants in the longitudinal studies to determine if the degree and rate of tissue loss is related to survival. The integrated measurement program will be continued. Repeat diet diaries will be collected for participants who participated in the nutrition survey begun five years ago. Additional participants will be reoriented into this aspect of the studies. Activity questionnaires will be collected at a rate which will provide an activity estimate within a few weeks of each diet diary.

Honors and Awards: None Publications: None

Serial No. HD-AG1(c)7
1. Gerontology Research Center
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Marital and Sexual Aspects of Aging

Previous Serial Number: HD-AG1 (c) (7)

Principal Investigator: C. Martin

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.94
Professional:	1.00
Other:	.94

Project Description:

Objectives: The present investigation is an attempt to: (1) secure measures of present and past aspects of marital and sexual behavior by which to compare longitudinal participants according to age and social characteristics, (2) delineate patterns of marital and sexual adjustment and their interaction, (3) seek evidence of correlation between measures of sexual functioning and physiologic and psychologic attributes among males of similar age, and (4) determine whether any aspects of marriage or sexual experience are related to the occurence of death or disease among males followed over time.

The desirability of pursuing multiple objectives in this manner is indicated by the close relationship between marital experience and sexual functioning, the few attempts to relate physiologic factors to differences in human sexuality, and because of the possibility that one or another type of sexual history may be significant for disease or longevity. As to the latter point, mortality data for each of the four color-sex groups in the United States, 1949-51, indicate that single, widowed and divorced persons exhibit death rates which are 1.1 to 3.9 times the rates found for the married. In addition, investigation has shown that breeders are shorter lived than non-breeders among mice, rats, cats, chickens and in Drosophila. Why these differences occur are unknown.

The present study is thus based upon the premise that carefully assembled

data on marital and sexual behavior may be variously utilized in the setting afforded by the longitudinal program.

Methods Employed: About half the number of participants returning to Baltimore for new testing in the past year were scheduled for interviews as time from other testing allowed. In requesting cooperation, the investigator cited his past research in human sexual behavior, advised subjects of the multiple objectives of the study, emphasized the voluntary nature of participation and provided assurance of confidence. In all, of 198 such requests to date, four participants declined to be interviewed.

Interviews followed a predetermined sequence of questions. As an aid to fluent communication, all questions were memorized along with their codes for recording responses. Chapters of the life history pertaining to residential, religious, military, marital, educational, occupational and parental-home experience were reviewed before taking up questions concerning marital adjustment and sexual conduct. This procedure of structuring the life history according to age, marital status, and other significant experience served to provide reference points by which both parties tothe interview were able to categorize behavioral events according to time.

Major Findings: Some 42, 39, 35 and 31 subjects falling into age groups 40-49, 50-59, 60-69 and 70-79, respectively, at interview reported that:

1. Marriage before the 25th birthday occurred in 43, 56, 26 and 19 per cent of cases in the respective age groups.
2. Coitus before the 25th birthday occurred in 81, 80, 51 and 42 per cent of cases in the respective age groups.
3. They had married once and were still married at interview in 91, 92, 86 and 68 per cent of cases in the respective age groups.
4. Coitus had occurred with two or more partners before the 40th birthday in 81, 72, 43 and 36 per cent of cases in the respective age groups.

Three types of evidence demonstrate a deterioration of sexual vigor with advancing age.

5. Subjects reporting intervals of less than a month in response to the question "At the present time, how long can you comfortably go without sexual activity of any kind. . . comfortably?" were 60, 49, 29 and 26 per cent of cases in the respective age groups.
6. The frequency of reported sexual activity for the five-year period immediately preceding the interview declined from 2.2 times weekly for subjects in their thirties to 0.5 times weekly for subjects in their seventies.
7. 81 males aged 40-59 and 64 males aged 60-79 at the time of interview, when compared with respect to quantity of sexual activity to orgasm over five-year intervals of the life span, show that those younger at interview were more sexually active than the older group over the period between 20 and 30 years of age but less active after age 35. The differences are consistent in the earlier onset of coitus and marriage described under items 1 and 2 reported above.

Males in the various age groups were also compared according to the total of reported experience to orgasm occurring between 20 and 40 years of age.

8. The data on quantity of sexual activity at ages 20 to 40 were divided into three categories and related to age at interview by means of a 4 x 3 contingency table. A chi square test revealed no significant association between such quantity of experience and age at report.

A comparison of data described under items 6 and 7 and the results of item 8 suggest a reasonably high degree of consistency within the data obtained. They also suggest (item 8) that quantity of sexual activity at ages 20 to 40 bears no particular relationship to longevity but that other factors found to be related to age should be further investigated by follow-up procedures.

Proposed Course of Project: Some 375 case histories will be available in another year at the present rate of accumulation. The larger series should better describe the range of individual variation which is found in all marital and sexual phenomena. A larger sample will also allow more extended inquiry into certain of the described objectives with an emphasis upon relating measures derived from the interviews to the physiologic, medical, social and psychologic attributes of individuals in collaboration with other staff of the Gerontology Research Center.

Honors and Awards: None

Publications:

Martin, C. E.: II. Marital and coital factors in cervical cancer. Amer. J. Publ. Hlth. 57: 803-814, 1967.

Serial No. HD-AG6(c)
1. Gerontology Research Center
2. Section on Cognitive Behavior
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Problem Solving and Age

Previous Serial No: HD-AG6(c)

Principal Investigator: D. Arenberg (45% time)

Other Investigators: None

Man Years:

Total	1.15
Professional:	.45
Other:	.70

Cooperating Unit: Baltimore City Hospitals

Project Description:

Objectives: Until recently, most efforts to explore human problem solving performance have been focused on end-products such as correctness of a solution and time to reach a solution. The processes involved in arriving at solutions, however, are of far greater interest and value in understanding problem-solving behavior. One reason for slow progress in this direction has been the paucity of methods which provide observable solution behaviors which can be objectively measured. Recent attempts to develop such methods have been fruitful, and two of these are described below and are used in this project.

One general objective is to determine in what ways age is related to problem-solving performance. Are age differences a function of difficulty or type of problem? If age differences are found, they can be explored further. For example, the differences may be due to difficulties of older people in acquiring the relevant information; or perhaps the differences are a function of the utilization of information (synthesizing acquired information). Are solution-strategy-preferences related to age? Even if no age differences are found

in the end results, there may be important age differences in how solutions are reached. The experiments in this project are designed to answer such questions.

Methods Employed: A modification of the Problem Solving and Information Apparatus (PSI) developed by John of Chicago is used in Experiment I. Each problem is displayed on a board with ten lights, nine of which have buttons to turn them on. The task of the subject is to light the light which has no button (the goal light) and this must be done in a prescribed way. There is a disk with a pattern of arrows for each problem. An arrow indicates that one of three logical relations (previously explained to the subject) exists between the lights connected by the arrows, but the specific relation is not known. Part of the problem is to determine the meanings of the arrows by inference or by questions of a prescribed form. The subject is instructed to infer as much information as he can in solving the problem. The ultimate solution of each problem is lighting the goal light by a sequence of inputs in which only a specified set of three of the nine buttons can be used. Time between inputs is controlled by the subject. There are many dependent measures possible for each problem in addition to whether the subject arrives at a solution and how many questions he requires, e.g., the number or proportion of questions asked about information inferable from the pool of information available at the time the question was asked. (This pool of information changes during solution.) This is one measure of redundancy, but others can be defined such as the number or proportion of questions or sequences of questions which are repeated (overt redundancy). In the two studies with PSI problems (Experiments I and IV), the subjects were participants in the longitudinal project.

In Experiment I, the subject was not required explicitly to identify the relation of each arrow on the problem disk. This is the procedure typically used in PSI problems. The subject may reach a solution with great difficulty when one or more of his implicit identifications of the relations is incorrect. This will sometimes appear to be a decrement in synthesis performance, when in fact the difficulty in putting the information together appropriately should be attributed to the misidentification which is an analysis error. To avoid the confounding of analysis and synthesis performance, Experiment IV was designed. What was a one-task problem in Experiment I was transformed into a two-task problem. The first task is to identify the relations (the meanings of the arrows between lights). After this is completed, the subject is provided with all the correct identifications and these are

reviewed before he is permitted to work on the second half the solution. As a result, the synthesis performance (solution) is always based on accurate and complete information, and is uncontaminated by poor performance during analysis (identification of relations).

Several studies of concept problem solving are also included in this project. In concept problem solving the subject's task is to identify a concept or the defining attributes of a set by using information from examples of what does belong to the set and what does not. Examples have various attributes and the solution is the group of attributes (concept) which defines the set and which determines whether an example is or is not a member of the set.

Experiments III and IIIa were concept-problem-solving studies with subjects whose education was average or below using simple problems (one attribute) and experimenter-selected examples. These have been described in previous reports. Experiment V is a concept-problem-solving study with high education participants (longitudinal). Conjunctive and disjunctive problems involving two foods as a solution are included as well as simple problems with one-food solutions. Examples are selected by the subject. The available gain in information can be measured for each example selected. To avoid fortuitous selections, the solution is not fixed. In this way, logically identical selections result in the same amount of information even though the specific selections are different. (Logically identical selections usually result in unequal information gain in problems with fixed solutions, hence the possibility of fortuitous solution).

Major Findings: For the 242 Ss in Experiment I with at least a college degree (ages 27-87) three problems (increasing in complexity) were scored. On the simplest problem , an increase in inferable questions (answers inferable from information available at the time the question was asked) was apparent only beyond age 65; prevalence of correct solutions also decreased beyond age 65. On the second problem, of intermediate difficulty, there was some indication of a decrement at age 55 and clearcut decrement by the sixties for both inferable questions and correctness. This occurred despite a decided selection factor operating due to the fact that several of the old Ss who performed poorly on the first problem did not have sufficient time to attempt the second problem (N=221). On the most difficult problem, there was a suggestion of a decrment at age 40, and a substantial decrement at 60 for the number of inferable questions. Again there

was an attrition in number of Ss (N=162) with the poorest performers on the first two problems failing to reach the third, and this may have accounted for the lack of an age deficit in prevalence of correct solutions. In two similar studies in the literature, overtly redundant questions were more prevalent among old than among young Ss. In this study, median numbers of overtly redundant questions were near zero for all age groups on the first problem. On the second problem, overtly redundant questions increased at 55 and were particularly prevalent beyond 70; and these data represent only correct solutions. On the third problems, a slight increase in overt redundancy appeared at 45, and increased again at age 60.

In Experiment III, a concept-problem-solving study with ten one-element (simple) problems, young Ss (N=21) correctly solved more problems than old (N=21) and this age difference was found for all five types of problems. The five types of problems were based upon the sequence of examples selected by the E for each problem. Each example could be positive (an example of concept) or negative (not an example of the concept) and some examples were redundant, i.e., provided no information not already available. Because a performance was obtained after each example, it was possible to identify the example which resulted in the "key" error for each problem solved incorrectly. It has been hypothesized that old Ss would commit more key errors than young immediately after negative examples and immediately after redundant examples, The results indicated that negative examples do not elicit more errors among old than among young men. However, those key errors resulting from redundant examples were made only by the old; no such errors occurred for the young group.

Experiment IIIa was a virtual replication of Experiment III; 31 old Ss have participated, but no young Ss have been available for this study.

No analyses have been made of the preliminary data in Experiment IV and V. A special-purpose-computor was constructed for Experiment V. The new apparatus provides the E with the proper feedback (whether the example selected is positive or negative) for each selection made by the S. Because there is no fixed solution, the proper feedback depends upon the relation between the information pool at the moment and the particular example selected. Before this apparatus was built, the E was required to figure out the appropriate feedback for each example selected by the S; now errors by E are minimized and scoring will be greatly facilitated by the apparatus as well.

Proposed Course of Project: Current plans are to prepare the results of Experiment I for publication. Logically identical problems have been prepared for longitudinal purposes, and data collection for repeat measurements will begin in the very near future.

Experiment II has been submitted for publication.

The search for a suitable group of Young Ss to complete Experiment IIIa will be continued.

Data collection for Experiments IV and V will continue.

Honors and Awards: None

Publications: None

Serial No. HD-AG7(c)
1. Gerontology Research Center
2. Section on Cognitive Behavior
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Verbal Learning and Age

Previous Serial No.: HD-AG7(c)

Principal Investigator: D. Arenberg (40% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.48
Professional:	.40
Other:	1.08

Project Description:

Objectives: The broad objective is to understand age deficits in verbal learning by studying experimentally those factors which influence age differences. Early studies explore age interactions with time intervals in rote verbal learning. More recent studies were designed to evaluate the effects of active responding, auditory input, decoding uncertainty, and the state (learned or unlearned) of adjacent items in a list on age differences in acquisition and/or retention.

Methods Employed: In Experiment X, a prompting condition was compared with the typical confirmation condition. Prompting refers to a procedure in which the inspection interval precedes the anticipation interval, the reverse of the usual order. This procedure increases the probability of active responding (correctly) during acquisition trials. The timeing of the intervals and display of the material was accomplished by a bank of five slide projectors controlled by a cam timer. In the self-paced test trials, one of the projectors was advanced by a manual switch after each response. Six acquisition blocks each consisting of twenty-four paired items, was followed by a self-paced test trial. Two hours after the

final test trial, a recall measure of retention was obtained on two more self-paced test trials. No feedback was provided on test trials. Subjects were low to average in vocabulary with no more than a high school education. A third condition was added to this study as a result of recent findings of the beneficial effect of passive auditory input in retention of digits, letters, and geometric forms. In this third condition, the E says the word aloud during the anticipation interval (when the letters only are in view), and the S is not instructed to respond during acquisition.

Experiment XII was a short-term-retention study with three input conditions: (1) visual only, (2) visual and experimenter says digits aloud, and (3) subject says digits aloud as they appear. A film of consonant sequences and digit sequences was prepared to provide 32 test items, 6 practice items, and 4 screening items at each of the three conditions. Each digit and each consonant was shown for 0.50 sec. (12 frames). The screening items consisted of a four-digit sequence and a short interval before writing the response. These items were used to determine whether the S could retain four digits when no interfering material followed. The practice items consisted of a three-consonant sequence followed by a two-second interval and another three-consonant sequence. The second group of consonants was said aloud by the S as they appeared; they served as interfering stimuli. The S was instructed to write the three consonants in the first group after saying the second group of consonants. The test items were similar to the practice items, with the exception that the first group consisted of a four-digit sequence and the second group consisted of a three-digit sequence. The three input conditions depended upon what occurred when the first group of digits or letters appeared. In the visual-only condition, the S looked at the first group as each element appeared. In the visual-with-auditory condition, the experimenter said each element of the first group aloud while the S looked at each element on the screen. In the condition with visual input and active responding, the S said each element of the first group aloud as the element appeared on the screen. Each S performed in all three conditions. A 16 mm movie projector externally modified with a monitoring synchronous motor provided precise timing of the visual display. A decade interval-timer was used to start and stop the projector to show a constant segment of film for each item. Primary comparisons were between an old and a young group with no more than a high school education. In addition, a group of old longitudinal participants (predominantly high in education) was included.

Experiment XIII was a short-term-retention study similar to Experiment XII. There was no interpolated material between presentation and response in Experiment XIII. The material consisted of six element digit sequences and letter sequences. A 16 mm film was prepared with twelve practice items (four-digit sequences, four-consonant sequences) and 48 main items (24 six-digit, 24 six-consonant). Three blocks, each consisting of 4 practice and 16 main items permitted each of the three input conditions to be used for each subject. The digit items were alternated with the consonant items throughout each film to reduce the interference from the previous items. Subjects had no more than a high school education, and were between 17 and 22 or between 60 and 70 years of age.

In experiment XIV, geometric figures were presented under two conditions: (1) the material was exposed for 12 seconds and the task was to reproduce the figures 15 seconds later; (2) the salient features of the figures were described during the 12 seconds they were exposed. Experimental subjects performed first under the visual conditions and then under the conditions with auditory as well as visual input. Control subjects performed twice under the visual conditions. A regression measure of change will provide a comparison between the two groups with considerable precision due to the within-subject design of the experiment. As in Experiment XII and XIII the hypotheses is that the performance difference between old and young groups will be decreased when the material is described (auditory input). Subjects did not have more than a high school education, and were between 17 and 22 or between 60 and 70 years of age.

Experiment XV is the first study of free recall. The procedure involves one presentation of 16 familiar one-syllable nouns which are not related and the task of the subject is to write all the words he can remember in any order. Proportion of words recalled will be related to position of the word in the presentation sequence. The list will be presented visually only or visually with auditory input. Primary interest is in relative effects on performance at the two ends of the list for young and old under the different input conditions.

Experiment XVI is a type of free-recall study designed to explore some aspects of interference due to items within the list (as contrasted with Experiment XI in which interference from another list was studied). According to a current hypothesis, the probability of retaining an item in a list should be greater if the immediately preceding item has been

learned than if the preceding item has not been learned; and similarily, if the following item has been learned. Preliminary training will provide a pool of learned words. Previously learned and unlearned words are then inserted into a free recall list in such a pattern that equal numbers of new words fall into four categories: (1) preceded and followed by learned words, (2) preceded by a learned word and followed by an unlearned word, (3) preceded by an unlearned word and followed by a learned word, and (4) preceded and followed by unlearned words. This will permit assessment of the separate effects of a learned word preceding and a learned word following and the combined effect of both. Data collection will begin with a young group, and if the results are promising, age will be included as a variable.

Experiment XVII was designed to study the effects of age and decoding uncertaintly on retention of nonsense syllables. Decoding uncertainty is a function of the number of ways a coded item can be decoded; the number of possible decodings depends upon the coding rule. For example, the syllable UFN can be coded into the word FUN to increase the likelihood of remembering UFN. If the coding rule is "rearrange the letters to form a word," FUN can be decoded into five possibilities, i.e., the other five possible orders of the letters FUN. If the coding is "move the vowel to the middle", FUN can be decoded into two possibilities, UFN or FNU. If the coding rule is "reverse the first two letters," UFN is the only decoding possible. Syllables are selected for each list so that all syllables can be coded according to one coding rule, and the greater the number of possibilities for that coding rule, the greater the decoding uncertainty. It has been reported that for college students, retention of syllables was inversely related to decoding uncertainty, and the proportion of decoded syllables retained to coded words retained decreased with increasing uncertainty in decoding. It is of interest to discover the effect of decoding uncertainty on old subjects, and to determine whether the proportion of decoded syllables to coded words retained falls more steeply with increased uncertainty for old than for young. Films have have been prepared to display for accurate time periods three lists of comparable syllables with identical coded words for all three lists.

Major Findings: In Experiment X, prompting was successful in increasing the frequency of active responding during acquisition for the old, but performance on the self-paced test trials was at least as high with the confirmation procedures as with

prompting for both age groups. Insufficient data are available for the third condition of Experiment X to determine the effects of passive auditory input (E says the response word during acquisition).

The results of Experiment XII, a short-term-retention study, showed that visual displays augmented by auditory inputs resulted in greater retention of digits than visual input alone. In addition, age differences decreased for the two auditory conditions. Although both young and old groups retained more items under active auditory (S says digits aloud) than under passive auditory (E says digits aloud), the age difference was not smaller under the active condition. Active responding did not benefit old more than young.

In an analysis of preliminary data from Experiment XIII, results were similar to those of Experiment XII. Experiment XIII is also a short-term-retention study with three input conditions, but unlike Experiment XII, no interpolated digits are presented. Greater retention for both auditory input conditions than for visual-only, particularly for the old, seems to be emerging.

In Experiment XIV, retention and reproduction of geometric forms is measured when the material is displayed visually and when salient features of the design are described while they are in view. Preliminary results indicate that old improve substantially when the auditory input is added.

In Experiment XV, list of words are presented once. Retention is best at the two ends of a list, but the preliminary data revealed no striking differences in input conditions for high shcool students.

Data collection has not begun for Experiment XVI and XVII, but the slides and films have been prepared, and the apparatus has been modified for these procedures.

Proposed Course of Project: Data collection will continue for experiments X, XIII, XIV and XV. Experiment XII will be presented at a national meeting, and a draft of a manuscript for publication has been prepared. Data collection will begin for Experiments XVI and XVII soon after the move into the new building.

Honors and Awards: None

Publications:

Arenberg, D.: Regression analyses of verbal learning on adult age at two anticipation intervals. J. Geront. 22: 411-414, October 1967.

Serial No. HD-AG8(c)

1. Gerontology Research Center
2. Section on Cognitive Behavior
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Relations Among Age, Time Estimation, Measures, and Time Judgment

Previous Serial Number: HD-AG8(c)

Principal Investigator: D. Arenberg (15% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	.35
Professional:	.15
Others:	.20

Project Description:

Objectives: Earlier objectives were (1) to determine whether age is related to simple time estimation measures (accuracy, variability, and context effects), and (2) to determine to what extent each of these four variables (age and the three time estimation measures) can account for performance on a more complex time judgment task involving interference from a set of interpolated stimuli. Similar judgment tasks have been found to be related to age in earlier work in this laboratory. More recent objectives were to determine whether age differences in interference on the complex time judgment task would be maintained under other experimental conditions; e.g., with no interpolated stimuli, labeling the interpolated stimuli differently, showing the interpolated stimuli without requiring judgments, presenting the primary stimuli via a different modality from the interpolated stimuli.

Methods Employed: Procedures for time estimation measures have been omitted because they are currently not being studied. Those methods have been described in previous reports.

The time judgment procedure varies, but in every procedure judgments of four stimuli (0.20, 0.35, 0.62, and 1.10 sec.) are made before and after an interval during which different events occur. Thirty-two judgments are made before the interval and the same number follow the interval. The primary data consist of the change in performance of discriminating the four periods before and after the interval. The conditions of

the experiment are determined primarily by what occurs during the interval. Usually twenty judgments of longer periods of light (1.10, 1.61, 2.14, and 2.68 sec.) are made during the interval. Under some conditions these periods have been labeled "one," "two," "three," and "four" which are the labels used for the main set of four periods. At other times, the interpolated periods have been labeled "four," "five," "six," and "seven" according to their ordinal position relative to the main set. Sometimes the interpolated lights are shown, but the S is not required to make judgments. In one procedure, no lights are shown during the interval between initial and final judgments. In another condition to be used, the interpolated periods will be presented auditorily instead of visually.

Major Findings: Results of three previous studies indicated that when the interpolated stimuli were labeled "one," "two," "three," and "four" the increase in errors following the interpolated judgments was consistently greater for old than young men. Recent results indicated that when no stimuli are shown during the interpolated interval no increase in errors occurs for young or old men, i.e., time alone does not result in a performance decrement.

Proposed Course of Project: Data collection will continue for the condition in which the interpolated stimuli are labeled "four," "five," "six," and "seven." If those data concur with previous results (greater interference for old than for young), the cross-modality conditions will be studied.

Honors and Awards: None

Publications:

Arenberg, D.: Retention of time judgment in young and old adults. J. Geront. 23: 35-40, Jan. 1968.

Serial No. HD-AG9(c)
1. Gerontology Research Center
2. Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Age difference in the control of plasma insulin concentration

Previous Serial Number: HD-AG9

Principle Investigators: R. Andres (30% time)
J. Avruch (10% time)
M. Haas (60% time)
L. Shenkman (40% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:
Total: 2.40
Professional: 1.40
Other: 1.00

Project Description:

Objectives: The long-range objective is to test the following two hypotheses dealing with the decline in glucose tolerance with increasing age: (1) decline in tolerance is caused by relative insensitivity of the pancreatic beta cell to several stimuli including arterial glucose concentration, and (2) decline in tolerance is caused by relative insensitivity of insulin-sensitive tissues to circulating insulin. A second goal is the development of a new physiological technique for the study in man of the glucose-insulin closed loop system, by extrinsic servo-control of the blood glucose concentration.

Methods Employed: (1) Insulin is assayed by a modification of the Morgan and Lazerow double antibody radio-immunoassay. (2) Plasma samples are obtained from the studies described in Project HD-AG10 and from the servo-control studies. (3) The servo-control technique has been previously described; in essence it involves adjustment in rate of a continuous intravenous glucose infusion according to the difference between the actual level of arterial glucose and the desired concentration.

Major Findings: (1) A significant improvement in the immuno-assay for plasma insulin has resulted in increased accuracy and greater capacity

through automation. The design and construction of a double-deck platter for the Sampler II of the AutoAnalyzer apparatus has made it possible to carry out the first step of the double-antibody reaction totally automatically. More importantly the separation of free and bound forms of insulin ^{131}I has also been automated. This step has probably been the source of greatest error in previous methods. It is now possible to run 120 samples per hour per step.

(2) Intravenous infusion of tolbutamide as a stimulus to pancreatic insulin release has been previously standardized for age. The mechanism of the decrement in performance on this test with age has now been further examined by study of the metabolism of the drug. Disappearance of the drug from plasma has been shown to follow a simple exponential disappearance after the initial mixing phase is over. This decay, expressed as "t 1/2", shows remarkable variability from subject to subject, ranging from 3.5 to 22.5 hours in a series of 26 subjects in the Gerontology Longitudinal Study Group. The subject's age did not influence this disappearance rate, but did have a significant effect on the plasma concentration of the drug 6 minutes after infusion. Surprisingly, the younger subjects showed the higher concentrations. Parameters which might be expected to influence this early concentration included (a) the tolbutamide distribution space, (b) the "efficiency" of the circulation (rate of distribution into the space), (c) drug metabolism (probably unimportant over such a short time interval). Older subjects would be expected to have tolbutamide spaces similar to that of young subjects since plasma volume and ECF volume do not change with age; tolbutamide space averages 14% of body weight, smaller than the ECF. Changes in serum albumin (to which tolbutamide is bound) with age might be a factor controlling the lower tolbutamide levels in the older subjects; this remains to be tested.

(3) A theoretical servo-control formula for the "glucose-clamp" experiments described in last year's report has been devised for a new set of studies on the effect of tolbutamide on pancreatic insulin release and synthesis. It is designed to maintain euglycemia despite release of endogenous insulin; thus the complex hormonal display in response to hypoglycemia can be prevented and the effect of tolbutamide per se can be evaluated.

Proposed Course of Project: Further studies of factors controlling plasma tolbutamide concentration will be carried out. Comparison of tolbutamide space and thiocyanate space will be carried out. The "glucose-clamp" formula for study of pancreatic beta cell responses to tolbutamide will be tested, and if successful, the effects of tolbutamide on insulin synthesis as well as release will be studied.

Publications: None

Honors and Awards:

1. Dr. Andres was an invited speaker at the Gordon Research Conference on the Chemistry of Aging at Santa Barbara, California, Jan. 24, 1968. Title: Homeostatic Mechanisms in Aging.

2. Dr. Andres was an invited speaker at the Research Training Program in Aging and Human Development at Duke University Medical Center, Apr.1, 1968.

Serial No. HD-AG10(c)
1. Gerontology Research Center
2. Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Age changes in carbohydrate metabolism in man

Principle Investigators: R. Andres (40% time)
M. Haas (25% time)
J. Avruch (40% time)
L. Shenkman (45% time)

Other Investigators: None

Previous Serial Number: HD-AG10

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	4.50
Professional:	1.50
Other:	3.00

Project Description:

Objectives: Diabetes mellitus is one of the leading causes of death and disability, but diagnostic standards for its detection remain arbitrary and controversial. There are four major tests for diabetes in common use. There is, however, little information concerning interrelations among these tests in the same subjects. Relative sensitivity (as judged by false negatives), and specificity (as judged by false positives) for these tests are not known. The present studies are designed to define standards of performance on these tests of a large group of active community-dwelling men covering the entire adult span of life. In addition, reasons for the decline in performance with increasing age are being investigated.

Methods Employed: Subjects are primarily those in the Longitudinal Study Group supplemented by college students and laboratory and hospital employees in order to increase the sample size of the group in the 3rd and 4th decades. Subjects are given one of the following tests per visit: oral glucose tolerance (OGTT), intravenous glucose tolerance (IVGTT), cortisone glucose tolerance (CGTT), intravenous tolbutamide response (TRT). On the fifth visit the cycle is re-started. Tests are performed under rigidly standardized conditions. Diabetic patients are available primarily from the Baltimore City Hospitals Out-patient Department; "chemical" diabetics and a small number of overt diabetics are available from the Longitudinal

Study Group.

Major Findings: (1) Since these tests were introduced into the test program in 1963, over 1700 tests have been performed, including 550 IVGTT, 550 OGTT, 250 CGTT, and 350 TRT. The complex interrelations among these tests and among other related variables in the test schedule requires computer technology. The NIH Computer Center has begun the task of programming and computation but definitive readouts are not yet available.

(2) The rapid intravenous glucose tolerance test is gaining favor as the preferred test of glucose tolerance especially among clinical investigators. Important technical factors influencing the apparently logarithmic decay of glucose concentration with time have been studied by comparing arterial, deep forearm venous, and superficial forearm venous plasma concentrations in 27 subjects. It was shown that the k value (per cent decline per minute) is significantly higher in the arterial samples than in either set of venous samples. Furthermore differences in k values between the two veins can be very large. The implication of these results is that the chance selection of forearm vein for blood sampling can introduce a significant technical variable - or indeed, error. In contrast, the striking similarity in the 60 minute glucose concentrations from the 3 anatomic sites suggests that the simple measurement of a single venous glucose concentration one hour after infusion may be a more reliable index of "tolerance" than the usual much more difficult collection and computation procedures.

(3) Studies of the plasma insulin response to the IVGTT have shown that pancreatic insulin release is rapid and that plasma concentration declines rapidly. Exact quantitation, however, of the early insulin changes has not been done thoroughly, and interpretation of the studies that have been done is difficult for two reasons: (a) glucose concentration in forearm venous blood does not accurately reflect either the true hyperglycemic stimulus (the arterial concentration) to beta cells and (b) changes in insulin concentration in forearm venous blood must inevitably also lag behind the concentration-time curves in the pancreatic vein, portal vein, central circulation, and finally in arterial (mixed) blood. In order to better define the beta cell response to hyperglycemia in man, a series of 50 experiments were done in which very frequent blood samples were obtained during the rapid increase in glucose concentration during a 2 (or 5) minute period of glucose infusion and during the subsequent 8 (or 5) minute period of rapid decline in glucose concentration as the injected glucose is distributed in its "space". Part of these studies had both arterial and venous samples, part of them had only venous samples. These frequent sampling studies showed that it is imperative to examine arterial levels if an accurate measure of the hyperglycemic stimulus to insulin release is desired, since the arterio-venous concentration differences are very large and very variable; prediction of arterial levels from venous concentrations is not feasible and should not be attempted. Other variables which were studied and which did not influence the time course of glucose concentration were (a) the volume of glucose solution injected and (b) the intravenous injection site. These variables were examined since the antecubital venous glucose concentrations curves during the first 10 minutes of the study showed remarkably varied patterns: some rose promptly and steeply, others showed

striking delays. Examination of the two variables above tested the hypothesis that a significant fraction of the infused fluid was pooling in the venous tree between the injection site and the central circulation. If this were true then the effect would be minimized (a) by infusing the same quantity of glucose in a larger infusion volume and (b) by infusing into the subclavian vein rather than into an antecubital vein. In fact these two variables did not influence the variance in the early part of the glucose concentration curve. These 2 studies show then that the variance in the venous glucose concentration curves is caused by variable circulatory lags in the peripheral circulation of the forearm itself and is not caused by venous pooling between the periphery and the heart. These studies confirm those which demonstrate that the k values derived from the later part of glucose curve (20 - 80 minutes) are higher in arterial samples than in venous samples: the forearm peripheral circulation is an important variable large enough to have clinical implications for interpretation of the k values.

(4) Previous studies had shown that sensitivity of peripheral tissues (the forearm) to insulin decreases with age, and that this may be a factor in the declining "tolerance" to glucose with age. The experimental technique is difficult however and the leucocyte was therefore turned to as a readily available "tissue" which might be utilized in the study of metabolic changes in man with age. Studies showed a surprising difference between the normal volunteer (Longitudinal Study) group and three groups of diabetic patients ("chemical" diabetics, overt diabetics on oral sulfonylurea therapy, and overt diabetics on insulin therapy): all 3 diabetic groups showed greater glucose utilization by WBC's than the control group. Furthermore, age of subjects had no effect on leucocyte metabolism. The differences between the diabetics and the control subjects could not be explained by differences in plasma insulin levels: neither endogenous insulin (plasma and leucocytes obtained one hour after ingestion of glucose load) nor exogenous insulin (100 or 1000 microunits per ml added to basal leucocytes) influenced glucose uptake.

Proposed Course of Project: The major achievement of the next year should be detailed analyses and comparisons of the four diagnostic diabetes tests. Correlations with serum lipids, dietary histories, activity levels, and anthropometric indices will be especially pertinant.

Publications:

1. Andres, R.: Diabetes and Aging. Hospital Practice 2: 63-7, Oct. 1967.

Honors and Awards:

1. Dr. Andres was an invited speaker at a Seminar on Medical Education sponsored by the American Geriatric Society, Feb. 19, 1968. Title: Physiological Factors of Aging Significant to the Clinician.

2. Dr. Andres was an invited speaker at a Postgraduate Seminar sponsored by the Summit Co. Diabetes Association (Ohio), Mar. 27, 1968. Title: Significance of the Glucose Tolerance Test.

Serial No. HD-AG41
1. Gerontology Research Center
2. Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Efflux of Free Fatty Acids from Isolated Adipose Tissue Cells

Previous Serial Number: HD-AG41

Principle Investigator: J. Avruch (50% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:
Total: 0.50
Professional: 0.50
Other: 0.00

Project Description:

Objectives: The process of lipolysis with adipose tissue can be divided into at least 2 phases, esterolysis and transmembrane flux of fatty acids. This study attempts to determine which of these processes is primarily involved as the site of action of certain agents.

Methods Employed: The assumptions underlying the study are: (1) Fatty acids, after esterolysis and prior to transmembrane flux exist in some intracellular pool.

(2) This pool is sufficiently large with respect to total intracellular free fatty acid, such that changes in the former will not be obscured by dilution in a very large sink of free fatty acids.

The rats utilized are in a known nutritional state - fed, fasted 24 or 48 hours. Isolated fat cells are prepared by the method of Rodbell. During the preparation of cells, glucose is added to the incubation mixture. The cells are then washed in glucose free medium and distributed in incubation medium of Krebs Ringer HCO_3 buffer with 2% fatty acid poor bovine serum albumin. Modifiers of lipolysis (ACTH, catecholeamines, insulin, prostaglandins, ouabain, dibutyrlcyclic AMP) are present, singly or in various combinations, with appropriate controls. The potassium concentration of the medium is to be varied in particular experiments. Samples (entire incubation mixtures, not aliquots) are taken at 0 time and every thirty minutes for 2

hours. The medium is separated from the cells after gentle centrifugation. The cells are washed twice with albumin free medium. The medium and washes are combined and analyzed for free fatty acids and glycerol. The cells are extracted with chloroform methanol, and FFA determined on this extract by titration. Cellular DNA is determined on the residue by the method of Kissane and Robbins.

Major Findings: Using fed rats of 200-250 gms, the basal intracellular concentration of FFA was 0.2 ± 0.1 μEq FFA/μg DNA. This rose to 0.4 ± 0.2 μEq FFA/μg DNA after one hour of incubation with ACTH, 1 mg/ml, and remained relatively steady for the next two hours. In the absence of ACTH, no significant change in ICFFA was discernable. The great variability in replicate determinations made interpretations of other experiments impossible. This variability was due to several factors. Most importantly, the washing and separation of the cells from medium after incubation led to losses of DNA and ICFFA which were not parallel. There also appeared to be an additional loss of DNA, varying from 5 to 30% during the lipid extraction. An attempt was made to separate cells from medium by millipore filtration. Studies with RISA in the incubation medium revealed that albumin contamination of the filter and thus the cells, amounted to 7-20% of the total RISA counts. This invalidated any measurement of ICFFA.

Proposed Course of Project: A method for cleaner and less traumatic separation of cells from medium is being sought.

Publications: None

Honors and Awards: None

Serial No. HD-AG12
1. Gerontology Branch
2. Nutritional Biochemistry
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Age Changes in Cellular and Tissue Biochemistry

Previous Serial Number: HD-AG12

Principal Investigators: C. H. Barrows, Jr. (1/3 time)
M. Schmukler (100%)
M. Epstein (85%)
M. Yiengst (100%)
J. Gregory (100%)
S. Leto (100%)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	7.18
Professional:	5.18
Other:	2.00

Project Description:

Objectives: The general purpose of this program is to examine various tissues for metabolic changes associated with senescence. Specific investigations during this period have included experiments to study the effect of age on (1) the metabolism of the brain, (2) metabolism of (2-^{14}C acetate) in liver, (3) sulfhydryl content of plasma albumin, and (4) renal clearances in the unanesthetized rat.

Methods Employed: (1) Standard techniques for the isolation and measurement of oxygen intake and of enzymatic activities of single cells and brain homogenates employing the Cartesian diver and Warburg apparatus. Brain cells have been grown in tissue cultures employing the Rose Chamber techniques. (2) Acetate metabolism has been measured by estimating the specific activities of glycogen, cholesterol, fatty acids, and proteins isolated by standard techniques following the intraperitoneal administration of (2-C^{14} acetate). (3) Plasma albumin was separated by the method of Pillemer and Hutchinson and the sulfhydryl determined by the method of Elman. (4) Catheters have been placed in the aorta, the right ventricle and in the

dome of the bladder of the rat. All catheters have been brought out subcutaneously to the dorsal cervical area. A collection cage has been designed and produced. By means of the above a constant perfusion can be maintained through the venous catheter, periodic blood samples can be taken through the arterial catheter; and the urinary bladder can be washed through the urinary catheter at the completion of a collection period.

Major Findings: (1) A new method of Cartesian diver micro gasometry has been developed which enables the measurement of carbon dioxide systems using a saturated $LiCl_2$ paraffin oil system. This has been first employed in the measurement of glutamic acid decarboxylase of brain homogenates and will be applied on a single cell level in studying spinal anterior horn cells. In addition preliminary work on trapping of radioactive gases has been successfully performed on gross samples of tissue and confirmed using the Warburg gasometer. The liberation of $C\text{-}^{14}O_2$ from glutamic acid is almost identical to the gasometric liberation of CO_2 by the enzyme glutamic acid decarboxylase found in brain homogenates.

Neurons, glia and connective tissue elements have been successfully cultured in vitro using the Rose Chamber system. The amount of tissue differentiation is quite gratifying and neurons, astrocytes, oligodendrocytes, ependymal cells can be identified. It has not been possible however, to culture brain tissue from rats older than four months of age.

Using standard gasometric analysis homogenates, prepared from prosencephalon, brain stem and cerebellum of 12 months, 2 months, and 26 months rats, have been analyzed for glutamic acid decarboxylase activity and the wet weights of the regions determined. Although there was no change in the enzyme activity between any age group, there was an increase in the brain weight of each region between the ages of 2 and 12 months.

(2) Statistically significant changes in incorporation into liver proteins between 3 and 12 months, and into fatty acids and glycogen between 12 and 24 months. Incorporation into protein and glycogen increased with age, while fatty acids decreased. It is planned to study these changes further and extend these studies to other tissues.

(3) The following correlations between -SH content of albumin and albumin content with age in 97 men were found:

ALB-SH	vs	Age	r= -0.54	$P < 0.001$
ALB	vs	Age	r= -0.26	$P < 0.01$
ALB	vs	-SH	r= +0.43	$P < 0.001$
-SH/ALB	vs	Age	r= -0.31	$P < 0.001$
-SH/ALB	vs	ALB	r= -0.41	$P < 0.001$

These data were compared with the data obtained by Yiengst and Barrows (see HD-AG12 1966-1967) on 68 male volunteers ranging twenty-six to eighty-one years of age. The data from the two groups was then combined and the following correlations were obtained on the total of 165 men:

ALB-SH	vs	Age	r= -0.60	P <0.001
ALB	vs	Age	r= -0.34	P <0.001
ALB	vs	-SH	r= +0.52	P <0.001
-SH/ALB	vs	Age	r= -0.36	P <0.001
-SH/ALB	vs	ALB	r= -0.30	P <0.001

The data obtained from female Wistar rats (10-12 month and 10-24 month rats) gave the following correlation:

-SH/ALB	vs	ALB	r= +0.28	ns

In comparison, the data of Yiengst on 53 rats gave the correlation:

-SH/ALB	vs	ALB	r= +0.435	P <0.001

Furthermore using the Student t-test for non-paried experiments and comparing -SH, alb, SH/alb; for the 12 and 24 month groups gave a significant difference between -SH contents at P 0.05 level. The age decrement in the SH/albumin support the concept that aging may be associated with the oxidation of readily oxidized compounds such as those which contain the sulfhydryl radicals.

(4) Values obtained in a limited number of rats for inulin clearance and TM^{PAH} are presented below.

	12 Month ♀ rat	24 Month ♀ rat	28 Month ♀ rat
N	4	4	2
inulin clearance ml/min/100 gm	.93 ± .18	0.90± .09	0.63 ± .02
TM^{PAH} mg/min/100 gm	.35 ± .02	0.34 ± .11	0.21 ± .01

Proposed Course of Project: The present work will be continued with a concentrated effort to study the enzyme changes in single spinal cord neurons from rats of different ages. In addition some effort will be made to quantitate the amount of lipofuscin in individually dissected motor neurons. An outgrowth of both these studies will be correlating enzyme changes with accumulation of lipofuscin pigment in single cells.

Once the life spans of the tissue culture colonies have been more definitely established biochemical analysis of single units will be undertaken at varying ages. This will offer an excellent opportunity to study glia since they are quite difficult to dissect directly from brain tissue preparations.

Finally it is hoped that the micro-radioactive trapping procedure can be scaled down for use with single cells. This will offer opportunities for biochemical analysis of single cells which essentially complement that of the Cartesian diver.

Continuation of the changes which occur in TPN-linked dehydrogenase isoenzymes in various tissues of rats of different ages.

Clearances on additional 12, 24, and 28 month old female rats will be performed.

Honors and Awards: None

Publications:

Hollander, J., and C. H. Barrows, Jr.: Enzymatic studies in senescent rodent brains. J. Geront. 23: (1), 174-179, 1968

Serial No. HD-AG13
1. Gerontology Branch
2. Nutritional Biochemistry
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Aging in the Rotifer

Previous Serial Number: HD-AG13

Principal Investigators: C. Barrows (1/3 time)
N. Meadow (100%)

Other Investigators: None

Cooperating Units: Dr. R. Herold, School of Dental Medicine
University of Pennsylvania
Baltimore City Hospitals

Man Years:

Total:	3.33
Professional:	1.33
Other:	2.00

Project Description:

Objectives: The rotifer has many characteristics which make it an excellent organism to serve as a model system to study biological aging. Among these are a short life span, parthenogenesis and poikilothermia. In addition it has been reported that nutrition and parental age influence the life span of this organism. The general purpose of this program is to determine the physiological, biochemical and histological characteristics of rotifers at various stages of their life span and to determine the basic biological mechanisms responsible for those factors which influence the longevity of these organisms.

Methods Employed: Specialized techniques for rotifer culture; and standard biochemical, radioisotope assay, and histological techniques, including electron microscopy.

Major Findings: (1) Culture and Longevity: To date, mortality and fecundity data has been collected from 796 rotifers grown under standard conditions (ad lib = feeding on A. aerogenes at 24° C). The mean life span of these animals was 22.6± 0.20 (S.E.) days, and they laid an average of 52.2± 0.21

(S.E.) eggs. These data agree well with those collected several years ago at the University of Pennsylvania on animals of the same strain grown under very similar conditions (lifespan = 22.2 ± 0.38 (S.E.) days, N=310; 52.9 ± 0.11 eggs/rotifer, N-106.)

Graded nutritional control of longevity has been achieved by diluting the bacterial nutrient medium with non-nutrient medium to obtain reduced bacterial counts/ml. Lifespan has been increased up to threefold by feeding 1/1000 the standard number (2×10^7) of bacteria.

Before attempting to select for strains of rotifers with different longevities, it was decided to test for the Lansing orthoclone effect in this species. Two orthoclones using the first eggs laid (pediaclones) were run, one for 50 generations, the second now in its fortieth generation. Neither of these orthoclones has shown an increase in longevity.

One orthoclone using the last eggs laid (geriaclone) was run for sixteen generations with no decrease in longevity. The only observed effect of orthocloning was on fecundity. Geriaclone animals laid fewer eggs than normal or pediaclone animals (46 eggs/animal compared to 52 eggs/animal); and they took longer to lay their eggs (geriaclones finished on day 14 - others on day 11.)

(2) Biochemistry: Techniques for measurement of the dry weight of a sample of about 50 rotifers, for estimation of microgram quantities of protein and RNA in rotifer homogenates, and for estimation of the lactate and malate dehydrogenase activity of single rotifers were applied to samples taken from a mass culture of rotifers (all animals of the same age).

The dry weight of a rotifer increased linearly from 0.35 to 0.70 ugm in the interval between the day of highest egg laying rate (day 7) and day 16, with no further increase by day 25 (the last day on which measurements were made.) The protein content of the rotifer was roughly 50% of the dry weight over the same period.

The RNA content per rotifer rose until day 11 and then fell. Based on a unit of dry weight or protein, however, the RNA content was highest at day 7 and then declined about 30% by day 25. Changes in lactate dehydrogenase activity per rotifer closely paralleled dry weight and protein changes until day 23, and then declined about 30%. Malate dehydrogenase activity per rotifer did not change so that on a dry weight or protein basis, there was about a 50% decline in activity between day 7 and 16. These data indicate marked biochemical changes associated with senescence.

(3) Histology: The last annual report mentioned that an electron microscope study of the stomach, dorsal gastric gland and ovary of the aging rotifer (in collaboration with Dr. R. Herold, University of Pennsylvania) revealed a decline in the number of ribosomes and mitochondria. Improved fixation techniques have revealed, in the same three organs, an age related accumulation of vesicles resembling lysosomes, which were not preserved in earlier experiments. Electron microscope histochemical reactions are now being used to test for the presence of lysosomal enzymes in the vesicles.

A crude quantitation of the accumulation of the vesicles was made on sections of dorsal gastric gland using a grid intersection technique. The vesicles comprised about 3% of the volume of the gland in young (6.5 day old) animals and over 30% of the volume of the gland in 20.5 day old animals. The accumulation in the stomach and ovary was not as extensive as in the dorsal gastric gland. The decline in ribosomes and mitochondria may be a result of replacement by the vesicles, but since the organs are growing, this relationship is obscured.

In a light microscope study of aging, a series of histological changes in the ovary was found to occur over the 24-48 hour period immediately following the production of the last eggs. The series consisted of a sudden coarsening of the granularity of the ovarian cytoplasm, and then a gradual loss of the coarse granules. In the electron microscope the coarse granules appear to be secondary lysosomes in the process of digesting ovarian cytoplasm, although no histochemistry has yet been done.

Proposed course of Project: (1) Culturing: An attempt is now being made to free the rotifers from contaminating bacteria and to develop a culture system with low risk of recontamination. If successfully developed, such a culture system will offer several advantages. First, it is hoped that eliminating bacterial contaminants will reduce the variability of lifespan. Second, it will make it possible to grow rotifers either on autoclaved bacteria or on completely artificial media. Enzyme assays performed on rotifers which are ingesting killed bacteria will be easier to interpret than in the case where live bacteria are ingested, because the killed bacteria will not contribute enzyme activity following autoclaving.

(2) Biochemistry: The experiments relating lactate and malate dehydrogenase activity to dry weight and protein content will be repeated to determine the reproducibility of the results. When these experiments are concluded, an attempt will be made to assay for lysosomal enzymes in rotifers from mass cultures. Also, an assay for DNA is being developed for use on mass cultured animals.

Preliminary experiments have shown age related changes in the rate of incorporation of labelled metabolites into RNA and protein. These observations must be repeated by more sophisticated techniques.

(3) Histology: Histochemical tests on the electron microscope level are being done to help identify the vesicles suspected of being lysosomes. To date, all animals selected for the electron microscopic study have been active, healthy looking animals. Dying rotifers will next be studied to determine if there are any changes in the vesicles during the death of the animal. Death is always preceded by several hours by a great reduction in movement, and by a shrinking and loss of optical density of the internal organs.

Enzyme histochemistry on the light microscope level will be used in conjunction with electron micoscope study, especially where no electron microscope test exists for an enzyme of interest.

After this, other organs, especially the brain, will be studied; and then, the effects of temperature and nutritional lifespan manipulation on the histological changes will be tested.

The fine structural changes in the ovary at the end of reproduction will be studied more closely, with the major aim of identifying the structures involved.

Honors and Awards: None

Publications: None

Serial No. HD-AG15
1. Gerontology Branch
2. Nutritional Biochemistry
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The relationship of nutrition to biological aging

Previous Serial Number: HD-AG15

Principal Investigators: C. H. Barrows, Jr. (1/3 time)
R. E. Beauchene (100%)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	3.33
Professional:	1.33
Other:	2.00

Project Description:

Objectives: To determine if the increased rates of synthesis of liver proteins and serum albumin observed in old animals, in vivo, is associated with proteinuria in these animals, or whether it is due to some more fundamental mechanism associated with biological aging, such as that suggested by the "Error Theory of Aging."

Methods Employed: Twenty-four hour urine samples were collected from young adult (11-15m) and old (24-30m) male rats. The urine was analyzed for total protein and globulin, albumin was obtained by difference. After the urine collections were completed, the rats were injected with acetate 2-^{14}C and tritiated isoleucine. After 30 minutes during which the rate of incorporation is linear the animals were sacrificed, the blood collected, and the liver and kidneys removed.

The specific radioactivities of the liver and kidney proteins and the serum albumin were estimated by liquid scintillation counting techniques.

Major Findings: The incorporations of acetate 2-^{14}C into liver proteins and serum albumin were higher in old than in young animals. The incorporation of H3 isoleucine into these proteins were not significantly increased in the old animals.

The specific activities of the total kidney proteins showed no age-associated differences. Although urinary proteins tended to be elevated in the old animals, the differences between the two groups were not statistically significant. The magnitude of proteinuria was not significantly correlated to the specific activity of either the liver protein or that of the serum albumin. It is felt that contamination contributed to the failure of being able to obtain statistical increases in proteinuria with age and to the non-significant correlations obtained between rates of protein syntheses and the magnitude of proteinuria in the individual animals.

Proposed Course of Project: We have improved our counting procedures for tritium by employing NCS as a protein solvent and eliminating spurious tritium counts by addition of formic acid to the scintillation medium. With these improvements in techniques, we are continuing the investigation and should be able to say conclusively whether the increased rates of protein synthesis observed in old animals, *in vivo*, is related primarily to proteinuria, and secondarily to age, or whether it is related to some other more fundamental mechanism.

Honors and Awards: None

Publications:

Beauchene, R. E., L. M. Roeder, and C. H. Barrows, Jr.: The effect of age and of ethionine feeding on the ribonucleic acid and protein synthesis of rats. J. Geront., 22: (3) , 318-324, July 1967.

Serial No. HD-AG21
1. Gerontology Research Center
2. Endocrinology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The renin-angiotensin system. Chemical-radiosotope derivative assay of plasma renin and angiotensin

Previous Serial Number: HD-AG21

Principle Investigator: Robert I. Gregerman (60% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.60
Professional:	.60
Other:	1.00

Project Description:

Objectives: The disease hypertension is, in reality, a conglomeration of distinct diseases with a common expression, namely, elevation of blood pressure. At least two of these diseases, primary aldosteronism and "ischemic" renal disease, are accompanied by abnormalities of the blood levels of renin, an enzyme whose origin is in the kidney. Measurement of renin is, in turn, of increasing value in the recognition of individuals affected by these variants of hypertension, an important first step in their therapy. A limit to progress in this area has been dependence upon cumbersome bioassay methods for the measurement of renin in blood, a fact which confines such measurements to a few research centers. Aside from its usefulness in the differential diagnosis of hypertension, renin is of central importance in the control of the adrenal's secretion of the steroid hormone, aldosterone. This effect is mediated through the action of renin on its plasma substrate to yield the peptide hormone, angiotensin. Thus, the measurement of renin has become an important part of the studies of the physiology and pathology of aldosterone.

The immediate goal of our work is the development of a radioisotope derivative assay for angiotensin and the application of this technique to the measurement of renin. Our secondary objective is the development of radioimmunoassay procedures for angiotensin. The latter approach will be applied not only to the assay of renin, as an alternative approach to the double isotope method, but to the direct measurement of angiotensin in plasma.

Methods Employed: Our previous reports have described in detail the chemistry of the double isotope derivative assay. Briefly, the angiotensin to be measured is converted to its ^{3}H-labeled denetrophenyl (DNP) derivative with high specific activity fluorodinitrobenzene. ^{14}C-DNP- angiotensin indicator is added to permit later correction for losses. Purification of the derivative is accomplished by thin layer chromatography. The method allows measurement of as little as a few nanograms of peptide.

Major Findings: Application of the double isotope method to the measurement of renin in human plasma yielded no measureable angiotensin II. Plasma blanks were low. The recoveries in control samples were adequate. Similar negative results were obtained whether endogenous renin was used or human renin was added to human plasma. An explanation for these results was quickly forthcoming.

We had standardized our double isotope method for the only angiotensin available, viz., asparagine^{1}-valine^{5} angiotensin II. The best evidence available until the fall of 1967 was that human angiotensin was identical to this material by chromatographic and electrophoretic criteria (Boncher, et al, 1961, 1964) and thus differed from the aspartic^{1}-angiotensin isolated from animal sources. Our method, as it was standardized, would not detect the latter.

In late 1967 Arakawa, et al, reported the first isolation and identification of human angiotensin. It appears to be the aspartic^{1} compound. We could not, therefore, have expected to measure angiotensin with our system.

For these reasons it became essential to standardize the double isotope assay in terms of human angiotensin. In addition, since angiotensin I (decapeptide) rather than angiotensin II is the first product of renin action, and since it has been recently demonstrated that conditions which prevent the destruction of angiotensin also prevent its conversion from angiotensin I to angiotensin II, standardization in terms of human angiotensin I is almost essential.

Although a synthesis of angiotensin I was recently published, its yield was very low and does not seem appropriate to provide sufficient material for our purposes. We have, therefore, undertaken a new synthesis of human angiotensin I and related peptides to provide the materials necessary for our further work.

The protected heptapeptide Arg(NO2)-Val-Tyr-Ileu-His-Pro-Phe(Benzyl) has been synthesized by the solid phase method. The impure product has been purified by preparative column partition chromatography. Reaction of this material with t-BOC-β-benzyl-aspartic acid-p-nitrophenlyester will yield the blocked octapeptide. Condensation of the latter by the carbodiimide method with His-Leu benzylester - which has already been prepared separately - will yield the blocked angiotensin I which, on hydrogenation, should yield angiotensin I.

Proposed Course of Project: The double isotope derivative approach to the assay of angiotensin and other peptides has been prepared for publication. When sufficient angiotensin I has been obtained, the double isotope method will be standardized with this material and the assay of plasma renin approached by this means. In addition, an attempt will be made to obtain antibodies to angiotensin I. Recent work elsewhere has resulted in a radioimmunoassay system for angiotensin II. Preliminary results of angiotensin blood levels, as measured with this technique, have been reported. However, assay of renin has failed, probably because the system generated angiotensin I with which the antibody to angiotensin II reacts only weakly. Furthermore, very recent work suggests that in the blood the predominant peptide is angiotensin I and that conversion may occur, not within the blood - as has been believed - but in the tissues. All of these considerations point to the urgency of establishing antibody systems for angiotensin I.

Honors and Awards:

1. Dr. Gregerman was invited to speak on "A double isotope derivative assay for angiotensin and other peptides" at a joint seminar of the Departments of Medicine, Physiology, and Pharmacology at Duke University, May, 1967.

Publications:

1. Gregerman, R.I.: Identification of histidylleucine and other histidyl peptides as normal constituents of human urine. Biochem. Med. 1: 151-167, 1967.

2. Gregerman, R.I.: Metabolic fate of histidylleucine, a metabolite of angiotensin I. J. Clin. Endocr. and Metab., May, 1968.

Serial No. HD-AG22
1. Gerontology Research Center
2. Endocrinology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Relationship of age to thyroid function and thyroid hormone metabolism

Previous Serial Number: HD-AG22

Principle Investigators: Robert I. Gregerman (40% time)
Paul J. Davis (85% time)

Other Investigators: Albert Dawkins*
Richard Hornick*

Cooperating Units: Baltimore City Hospitals
*Division of Infectious Diseases,
University of Maryland
School of Medicine

Man Years:

Total:	2.25
Professional:	1.25
Other:	1.00

Project Description:

Objectives: Earlier studies from this laboratory have established an age-related decrease in human thyroid gland function which is secondary to a slowing or hormone (thyroxine, T4) disposal (turnover). The mechanism of this change is not understood. In order to gain an understanding of the factors which control hormone turnover, and hence the basis of the age-related change, we elected to study in detail a situation in which we have shown the occurence of rapid changes of T4 turnover, viz., acute infectious illness. Several major points were examined:

A) The concept that plasma "free" (non-protein bound) T4 determines T4 disposal rate.
B) The hypothesis that acute change in the concentration of plasma thyroxine binding pre-albumin (TBPA) in turn regulates plasma free T4 concentration.
C) The contribution of acute changes of protein-bound hormone concentration (total serum thyroxine or plasma PBI) to the observed acute changes of free thyroxine.

Additional closed related studies included:

1) A re-evaluation of the quantitative role of TBPA in the transport of T4 in human plasma.
2) Observations on the thyroxine binding globulin (TBG) of familial TBG deficiency.
3) Acrylamide gel electrophoretic observations of the plasma protein carriers of T4 in the rat, as a basis for detailed studies of factors affecting plasma free thyroxine in an animal model system.

Methods Employed: Free T4 was determined by equilibrium dialysis; TBPA and TBG by saturation techniques with paper electrophoresis; T4 turnover by plasma disappearance of ^{125}I-T4. Additional methods included electrophoresis of T4 labeled serum in agarose and polyacrylamide gels.

Fifteen prisoner volunteers at the Maryland State Penitentiary served as subjects for the infectious disease studies. Five has tularemia, another five had malaria, and the third group had typhoid fever.[1] Simultaneous serial daily measurements of TBPA, TBG, free T4, and T4 turnover were made during control periods, during symptomatic infection, and in the period of recovery. Each study lasted 3 to 6 weeks.

Major Findings: Preliminary results of the infectious disease studies were first reported last year. The measurements have now been completed and are summarized as follows:

A) Free T4 and T4 turnover: Three of the five subjects with tularemia had acute increases of free T4, as did 4 of 5 subjects with typhoid fever, and all of the subjects with malaria. All of the 5 subjects with tularemia and 4 of the 5 with typhoid showed absolutely no alteration of T4 turnover. In only one case of typhoid did T4 turnover increase briefly, but in this instance turnover reverted to normal even as free T4 remained elevated. In two of the cases of malaria T4 turnover accelerated after free T4 rose, but reverted to normal or slower than normal rates of turnover even as free T4 remained elevated.

   These observations show a complete dissociation between T4 turnover and alterations of free T4: free T4 may rise and fall while T4 turnover is unchanged; turnover may accelerate and slow while free T4 remains elevated. We have concluded from these observations that acute change of free T4 is not a determinant of T4 turnover. The possibility remains that chronic elevation of free T4, as in hyperthyroidism, may contribute to the acceleration of T4 turnover under those circumstances.

B) TBPA as a regulator of free T4: A rapid fall of TBPA was coincident with clinical illness in almost all cases. The fall was roughly correlated with the severity of the clinical picture.

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1. These studies were the responsibility of the University of Maryland.

While free T4 often rose as TBPA fell, close examination of the time relationships showed marked dissociation. In general, TBPA fell to low levels before free T4 rose, while free T4 often remained elevated even as TBPA returned to its normal level. In one instance TBPA fell and rose with no change whatever in free T4. In another case free T4 rose and fell while TBPA was unaltered. These observations are apparently incompatible with the view that TBPA is responsible for the observed changes in free T4.

C) Changes in blood hormone concentration (PBI): In most instances there was no consistent change of the PBI, but in two cases marked alteration was seen, as well as lesser change in several others. The pattern was for a fall of PBI early in the illness, followed by a rise to as much as twice the value at the nadir. In these cases the observed changes of free T4 were at least partially secondary to changes of PBI. In one case doubling of the free thyroxine during illness was entirely related to a change of the PBI.

Conclusions from these studies: These observations seem to establish that, contrary to a long held view, thyroxine turnover is not directly determined by plasma free thyroxine. Furthermore, thyroxine turnover is independent of febrile illness, per se, and the hypermetabolism which accompanies such illness. Alteration of thyroxine turnover when it does occur, seems better related to the specific disease responsible. Our previous observations showed a high incidence of acceleration of turnover in bacterial pulmonary infections and a similar situation exists in malaria. On the other hand, acceleration occurs only rarely in typhoid fever and not at all in tularemia.

Free T4 has been shown to change serially in infectious illness, as has been shown earlier by others with surgical stress, but the present studies show that free T4 varies quite independently of TBPA. These observations appear to refute the widely held view that acute changes of free T4 are secondary to alterations of TBPA. Some factor other than TBPA is probably responsible, perhaps an acute phase component which interfers with thyroxine-protein interaction.

Finally, acute illness occasionally results in changes of plasma thyroid hormone concentration (total T4), and via this mechanism, is occasionally and soley responsible for acute alterations of plasma free T4.

Other Related Studies:

1) TBPA as a plasma T4 carrier protein. These studies grew out of observations described above which suggest that TBPA, contrary to current views, is not an important carrier of plasma T4 under physiologic circumstances. Data available from the literature indicated that 30% of plasma T4 is bound to TBPA. We re-examined this problem using electrophoresis of serum containing tracer a-

mounts of ^{131}I-T4 in agarose gels.

The following results were obtained:

a) At pH 7.4 in normal male plasma 13.7 ± 1.1%(SD) of the T4 is bound to TBPA. The remainder is bound to TBG and albumin. This value is mcuh lower than those obtained earlier by others.
b) The binding of T4 to TBPA is dependent on pH. At pH 8.6 nearly twice as much T4, 25.4 ± 1.8%(SD), is bound to TBPA as at pH 7.4. The pH dependence has not been previously recognized.
c) At 31°C about 1/3 more T4 is bound to TBPA than at 37°C.

We concluded from these observations that TBPA is probably only a minor carrier of T4 in human serum under physiologic circumstances. We now believe that previous data which suggested that TBPA carried considerably more T4 at physiologic pH were in error because of failure of previous workers to control pH. Our results have been communicated to the original investigator in this area (C.S. Hollander) who has now repeated his original observations. As we have suggested, he found that pH was in error in the original experiments, and has confirmed our present results exactly.

2) Observations of TBG in familial TBG deficiency. We have explored the possibility that the decreased TBG seen in this condition is due not simply to a decrease of binding protein concentration, but to some quantitative abnormality of the binding protein. Initially we believed that this TBG binds T4 at pH 8.6 but not at physiologic pH. Those observations, made with cellulose acetate electrophoresis, were shown to be artifacts of the electrophoretic medium. A polyacrydamide gel system was developed in a pH of 7.4 which can be maintained (piperazine buffer) and which resolves TBG, albumin, and TBPA. TBG from the cases with low TBG binding capacity, as conventionally measured, shows neither alteration of electrophoretic mobility nor any differential effect of pH on T4 or T3 binding.

   The gel electrophoretic system developed for this specific purpose is now being used to measure for the first time the effect of pH on the maximal binding capacity of TBG which has heretofore been measured only at alhaline pH (8.6). This system should also be useful in studies of drug-TBG-T4 interactions which also have been previously impossible to study at physiologic pH.

3) Plasma protein carriers of T4 in the rat. The desirability of an experimental model for studies of factors influencing acute alterations of free T4 has led us to reexamine the characteristics of the T4 binding system in the rat. Preliminary acrylamide gel studies suggest the presence of three carrier proteins, the major one of which appears to be pre-albumin.

Proposed Course of Project: The results of the studies on changes of T4 turnover, TBPA, and free T4 in infectious illness are being prepared for publication. The studies of TBPA binding of T4 at physiologic pH have already been prepared and cleared for publication. A paper dealing with the chromatographic purification of radioactive thyroxine and triiodothyronine is being prepared for publication. The observations on the TBG of familial TBG deficiency are being completed and will be prepared for publication, along with a description of the effect of pH on TBG binding capacity and the new electrophoretic system. The nature and characteristics of the T4 binding proteins of the rat will be explored in detail (binding capacities, pH effects, etc.) as a preliminary to further observations on the control of free thyroxine in plasma.

Honors and Awards: None

Publications: None

Serial No. HD-AG24
1. Gerontology Research Center
2. Cardiovascular Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Role of Hemodynamic, Metabolic and Hormonal Factors in the Performance of the Aging Rat Heart

Previous Serial Number: HD-AG24

Principal Investigators: D. Shreiner (100% time)
M. Weisfeldt (85% time)

Other Investigator: N. W. Shock

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	2.51
Professional:	1.85
Others:	.66

Project Description:

Objectives: The initial phase of this study has established an age and sex difference in the work performance of the isolated rat heart. Although these data do suggest certain mechanisms to explain this age difference in performance, no firm conclusion can be drawn. With these data in mind, an effort has been made to design preparations in which the effect of age on particular aspects of performance can be studied. These aspects include: (1) the intrinsic contractile state of the myofibrils (contractility), (2) the left ventricular chamber size and muscle mass, (3) the size of the effective coronary vascular bed, (4) the oxygen extraction ability of the myocardium, and (5) the response of the contractile elements to hormonal and pharmacological factors.

Methods Employed: A. The isolated blood-perfused rat heart -lung and heart preparation.

These preparations have been described previously in detail. In the heart-lung preparation, oxygenated rat blood was circulated through the right and left side of the heart and lungs

before returning to the oxygenator. In the isolated heart preparation, blood was circulated only through the left side of the heart before returning to the oxygenator. In this case blood collecting in the right side of the heart was measured as coronary blood flow and then returned to the oxygenator. Aortic and coronary blood flowwere measured and blood pressure, heart rate and temperature were maintained at a constant level.

B. The isolated heart preparation perfused without red blood cells.

In this preparation the heart was perfused in a non-recirculating fashion with a modified Krebs-Ringer bicarbonate solution. This basic solution was modified by reducing the calcium and magnesium concentration to one-half and by the addition of: (1) 3.26% Dextran molecular weight of 76,000 (an oncotic agent), (2) 300 mg% glucose, (3) 0.5 mM di-calcium-EDTA (to eliminate a vasoconstrictive factor thought to be due to heavy metal contaminants). The perfusate was filtered through a millipore filter (3 μ pore size) and maintained at a constant temperature of 36-37°C. Perfusion was done by the Langendorff (retrograde aortic) technique. The heart was paced at a constant rate above its intrinsic rate (320 beats per minute). In order to insure that the heart was essentially non-working, a small catheter was inserted into the left ventricular apex in order to decompress the left ventricle. Cardiac performance was measured by recording isovolumic left ventricular pressure after insertion of a fluid filled latex balloon into the left ventricle via the mitral valve orifice. The heart was placed in a small chamber filled with fluid and the outflow from the chamber was used to measure coronary flow and venous oxygen content. This preparation has the following attributes: (1) constancy of perfusate composition throughout the experiment, (2) the elimination of particulate matter from the perfusate, (3) stability of performance. Its major disadvantage is its high coronary flow. Also, the question as to the adequacy of myocardial oxygenation under working conditions is presently under investigation.

C. The isolated rat heart preparation perfused with "simplified" blood.

This preparation, which is presently in its initial stage of development, will be similar to the non-blood perfused preparation except for the use of washed erythrocytes in the perfusate solution and the use of a glass wool filtration system. Hopefully, the use of the glass wool filter, non-recirculation, and the elimination of platelet and leucocyte aggregates and proteins will prevent the formation of micro-emboli and eliminate the progressive rise in coronary vascular resistance seen in the recirculating blood-perfused preparation. The main

advantage of this preparation lies in the ability of the washed erythrocytes to provide adequate oxygen delivery to the myocardium without contending with the problems of whole blood.

Major Findings:

1. Age and sex differences in the total work performance of the isolated blood-perfused heart-lung preparation. It was previously found that the hearts of senescent male rats performed less total work and had a shorter survival time than the hearts of young adult male rats. Under the same conditions, the hearts of young and old virgin female rats showed no age difference in their work performance.

2. The effect of breeding status on the work performance of the heart-lung preparation. In contrast to the high work performance of the virgin female rats, the hearts of female rats that had been used for breeding performed poorly in the heart-lung preparation. Although the hearts of the young ex-breeder females performed somewhat better than the hearts of old ex-breeder females, this difference was not significant. The work performance of both age groups of ex-breeder females was similar to that observed in the old male heart.

3. The possible role of coronary blood flow in the observed differences in work performance. In the blood-perfused isolated heart preparation coronary blood flow was measured in the hearts of young and old male rats. Although the cardiac output was not significantly different between young and old hearts near the beginning of perfusion, the coronary blood flow of the old hearts was lower than that of the young heart. The coronary blood flow remained lower in the old than young heart throughout the experimental period. As in the heart-lung experiments, the total work performance of the isolated heart was lower in the old than the young male hearts.

4. The possible role of ventricular size in these differences. The hearts of male rats weighed about 30-35% more than the hearts from female rats of comparable ages. No significant difference was found between the wet weights of hearts from young and old virgin female rats. However, the mean wet weight of the hearts of old ex-breeder females was about 10% greater than that of the young ex-breeder females. The hearts of old male rats weighed about 8% more than the hearts of young male rats.

Although the left ventricular wall thickness of the old male hearts was not significantly different from that of the young male hearts, the left ventricular chamber was about 37% larger

in the old than in the young male rat hearts. These results show that dilatation of the left ventricle occurs to a greater extent than does hypertrophy in the aging male rat heart. In the female the size of the left ventricular chamber did not change with aging. This was true for hearts of both virgin and ex-breeder female rats, despite the fact that there was a slight increase in weight of the left ventricle in the older rats.

5. Properties of the non-working isolated heart preparation perfused without erythrocytes. This preparation had a stable coronary blood flow and oxygen consumption for periods up to 90 minutes or more. In contrast to this, the same preparation without dextran in the perfusate showed a significant decline in these parameters during the first 45 minutes of perfusion. These hearts were able to autoregulate coronary blood flow to a substantial degree between perfusion pressures of 50 to 90 mm of mercury. There was a small (10%) rise in oxygen consumption with this increase in perfusion pressure similar to non-working preparations of hearts from other species. In response to a lowering of arterial oxygen content, there was a parallel increase in coronary blood flow and oxygen extraction. Oxygen consumption remained constant until the arterial oxygen content fell to such an extent that compensation by increased oxygen extraction and by increased coronary blood flow was insufficient to maintain oxygen consumption. Adequate oxygen availability is assumed to be present at an oxygen content above this "critical" level.

Summary and Conclusion: The hearts of old male rats have a lower total work performance and a lower coronary blood flow than the hearts of young male rats. Although there is no age difference in the work performance of hearts from virgin female rats, the performance of hearts taken from both old and young ex-breeder female rats is much lower than that of the old or young virgin female rats. The role of coronary blood flow as an etiologic factor in the performance of these hearts has not yet been defined. The fact that the hearts from old male rats have a larger left ventricular chamber, and therefore, more wall tension at a given pressure than the hearts of young male rats suggests that dilatation in the old male heart may be a factor in their poor performance. This mechanism cannot explain the poor cardiac performance of both young and old ex-breeder females, since their chamber size was not different from that of the virgin female rats. The performance of the hearts from ex-breeder rats is presently under investigation.

The development of a stable isolated heart preparation that does not need to be perfused with blood has provided a means for further studies of coronary flow and performance. Hearts perfused with a modified Krebs-Ringer's solution with added

glucose and dextran remain stable for a significant length of time and are able to alter their coronary flow and oxygen extraction so as to maintain a constant oxygen consumption.

Proposed Course of Project: A. Further development of preparations for the study of specific aspects of aging in the rat heart: the isolated rat heart perfused with simplified blood should provide data as to the oxygen consumption and performance level of the isolated rat heart under conditions of adequate oxygen availability. These results will be compared with those from the same preparation without erythrocytes in the perfusate. Depending upon the results of this comparison, one of the preparations will be chosen to be used to study the effect of age on the mechanical function of the isolated rat heart.

B. The young and old rat hearts will be compared under the conditions described above in terms of the maximum isovolumic systolic pressure at various left ventricular and diastolic volumes, pressures and levels of estimated diastolic wall tension. Maximum isovolumic pressure will be converted to estimated wall tension so that comparison between rats of different ages and heart sizes can be made. The oxygen consumption at various levels of performance, the dp/dt, and time to peak tension will also be studied with respect to age. Analysis of velocity differences will be complicated by the lack of knowledge about age changes in the series elastic component, but one will be able to evaluate the resultant effects of any age change in series elastic component and contractile element velocity with respect to the function of the ventricle as a whole.

C. The coronary vascular bed will be studied in old and young rats using the non-working isolated rat heart preparation which is perfused with a medium free of erythrocytes. The vasodilating capacity will be determined by measurement of coronary flow during progressive lowering of the arterial oxygen content. Also, under the same conditions, information will be obtained as to the maximum ability of the young and old heart to extract oxygen from the perfusate. (That is to say the amount of functional arteriovenous shunting.) In addition, an estimate of the effective coronary flow per unit of myocardial mass will be made.

D. Alterations in myocardial metabolism and hormonal responsiveness with aging will be the next broad area of approach after the completion of the above studies. The preparations described should allow an opportunity to study the comparative response of the myocardial contractile elements to hormonal factors and induced changes in myocardial metabolism.

Interpretive Statement

These studies suggest that dilatation of the left ventricle is a significant mechanism in the poor work performance of the aged male rat heart. However, whether dilatation is a primary aging process or is secondary to some other process is not yet known. Cardiac dilatation is usually a secondary manifestation of some disorder such as valvular insufficiency, increased outflow resistence, or coronary artery disease. There is no evidence perhaps coronary artery disease.

Our data show that at the same work load, the old male heart has a lower coronary blood flow than the young male heart. Other investigators have noted periarteritis involving many vessels, including the coronaries, in older rats and a kind of arteriosclerosis has been described in female rats used for breeding purposes.

If coronary artery disease is responsible for the ventricular dilatation seen in old male hearts, why was dilatation not found in the hearts of female ex-breeder rats? These hearts from young and old ex-breeder rats demonstrated a poor work performance in the heart-lung preparation. It is possible that the coronary artery disease seen in ex-breeder rat hearts is responsible for their low work capacity, but did not cause dilatation because of the lack of some predisposing factor or factors that perhaps did exist in the aging male rat. Since a sex difference in myocardial work performance does occur in the rat, one would think that the sex hormones might be one of these factors. One could postulate that an excess of female hormones as occurs in pregnancy leads to deleterious effects on the coronary arteries, but that the levels of hormones in virgin female rats has either no effect or a protective effect on these vessels. In contrast, the male sex hormones could either have a deleterious effect or lack some protective effect on the coronary vessels. The role of coronary blood flow in the performance characteristics of the aging heart is still under investigation.

The major goal of future work in this area is to provide experimental designs in which specific aspects of the total cardiac performance can be considered individually. This can best be done under conditions of stability, i.e., with the use of a preparation whose function in forms of the parameters being studied is not declining. At present we have available preparations which will allow selective study of the functional capacity of the coronary vascular bed and selective study of contractibility of the myofibrils as reflected in isovolumic left ventricular performance.

Honors and Awards: None

Publications: None

Serial No. HD-AG42
1. Gerontology Research Center
2. Cardiovascular Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Distribution of Vitamin E in Beef Heart Mitochondrial Fractions; Quantitation of Vitamin E in Organs of Young and Old Rats; Vitamin E Deficiency histopathology in the Rat.

Previous Serial Number: HD-AG42

Principal Investigator: W. B. Weglicki (100% time)

Other Investigators:
A. Nason
W. Reichel
P. P. Nair
N. W. Shock

Cooperating Units: The Johns Hopkins University and Sinai Hospital, Baltimore

Man Years:
Total: 1.2
Professional: 1.0
Others: 0.2

Project Description:

Methods Employed: (A) The distribution of vitamin E within the mitochondria of heart muscle has been pursued by using the Hatefi method of deoxycholate disruption followed by ammonium acetate precipitation of DPNH and succinate cytochrome c reductase fractions; vitamin E was found in the non-saponifiable lipid extracts, purified by silicic acid column chromatography and quantitated by gas-liquid chromatography. (B) In 12 and 24 month old rats the heart, liver, brain and adrenals were extracted by lipid solvents and (by the methods in A) vitamin E levels determined. (C) A group of 3-4 month old female rats was maintained on a vitamin E deficient diet for 12 weeks; a control group was fed the same diet to which 50 U of vitamin E/Kg was added. Pigment fluorescence was measured in unstained sections using a Zeiss photomicroscope equipped with a grid superimposed on the microscopic field.

Major Findings: The levels of vitamin E found in beef heart mitochondria varied from 111.1 to 115.6 μg/gm protein. Table 1 lists the mean levels of vitamin E found in young and old rat organs. Table 2 shows the % pigment found in the E deficient and sufficient adrenal glands.

We have confirmed the concentration of vitamin E in beef heart mitochondria and have demonstrated the distribution to be throughout the various ammonium acetate fractions rather than in association with a particular enzyme fraction. The work in progress indicates an age and sex related elevation of rat tissue vitamin E; further analyses are in progress to investigate this result. The quantity of lipofuscin-like pigment in the zonareticularis of E deficient adrenals of 3-4 month old female rats approaches that found in 12 month old female rats. This result is being studied in a similar group of male rats. Tissue levels of vitamin E are also being determined to demonstrate the extent of deficiency in these animals.

Proposed Course of Project: The project will be terminated in June since Dr. Weglicki's tour of duty in the Public Health Service will be completed.

Honors and Wards: None

Publications: None

Table 1

Average values of tissue vitamin E content in 12 & 29 month old animals

	12 month old		29 month old	
	Male	Female	Male	Female
Brains µg	178 (137-243)*	300 (138-390)	147 (90-198)	262 (232-287)
Livers	23 (12-42)	33 (25-45)	79 (70-93)	105 (80-125)
Hearts	6 (<1-14)	25 (<1-52)	9 (<1-15)	18 (9-38)
Adrenals (pooled)	686	376	1407	1495

* Numbers in brackets indicate range of values in 4 individual determinations.

Table 2

Lipofuscin-type Pigment Accumulation in Vitamin E Deficient Female Wistar Rats vs. Vitamin E Sufficient Control Rats

	Deficient	Sufficient	
Age (Mo.)	(3.5)	(3.5)	
Number	7	7	
Location	% section vol. occupied by pigment		(P value)
Heart (left vent)	0.0 ± 0.00*	0.0 ± 0.00	N.S.
Adrenal Cortex (z. glomerulosa)	0.0 ± 0.00	0.0 ± 0.00	N.S.
(z. fasciculata, outer 2/3)	0.0 ± 0.02	0.0 ± 0.00	N.S.
(z. fasciculata inner 1/3)	0.2 ± 0.10	0.0 ± 0.00	N.S.
(z. reticularis)	2.9 ± 0.28	0.4 ± 0.14	<0.001
Adrenal Medulla	0.1 ± 0.06	0.0 ± 0.00	N.S.

* Mean ± SEM

Serial No. HD-AG25
1. Gerontology Research Center
2. Physiological Psychology
3. Baltimore, Maryland 21224

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Operant responding, maze learning and retention in rodents as a function of age

Previous Serial Number: HD-AG25

Principal Investigator: C. L. Goodrick (60% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	1.20
Professional:	.60
Others:	.60

Project Description:

Objectives: Behavioral genetic research during the past decade has clearly shown that variables such as learning, memory and activity are determined in part, through inheritance. One aspect of this program is to analyze the contribution of inheritance to changes in behavior due to aging.

Another phase of this program is to further identify the behavioral changes which occur during aging, to identify the mechanisms which mediate these changes and to devise techniques for overcoming them.

Methods Employed: Animals are tested in operant conditioning chambers or mazes under conditions which have been standardized in this laboratory. They include maze learning, operant level, light contingent bar pressing, operant responding for food or water rewards and extinction while non-deprived, food and/or water deprived. In studies with genetically pure strains of mice, differences in performance due to aging have been analyzed in short lived (A/J), long lived (C57BL/6J) and F_1 hybrid groups.

Major Findings: 1. A behavior genetic experiment was completed to determine the feasibility of studying operant performance and retention by means of light contingent bar pressing (LCBP) responses in a 2-bar test box. Subjects were inactive, albino A/J mice, active, pigmented (black) C57BL/6J mice and an F_1 cross (groups A, C, and F respectively). All mice were

young males. The mice were tested to determine (a) operant level, (b) rate of responding and proportion of LCBPs (one bar was light contingent with the other neutral) during continous reward, fixed ratio reward for every fourth response (FR 4) or FR 7, and (c) rate of responding and proportion of responses on the formerly rewarded bar during extinction. During operant level tests C mice responded more than A mice while F mice displayed heterosis, i.e., they responded more than either parental group. Initially, the same relations occurred for average response rates during LCBPing, but when the FR requirement was increased, differences between all groups were reduced. Group differences were not statistically significant for LCBP proportions. During retention tests, A mice made a higher proportion of reward bar responses than C mice, but C mice maintained a higher rate of response than A mice. Mean response rates of hybrid mice during extinction were similar to those of C mice, while reward bar proportion means were similar to those of A mice. F mean proportions tended toward intermediate inheritance with increasing trials. These data provide information concerning the inheritance of the retention of responses as well as a research model with implications for the study of aging. Specifically, variables which act to change response scores of inactive A mice, may be utilized in the attempt to change response patterns of senescent rodents.

2. Young and senescent, food-deprived rats were tested for operant responding during fixed interval (FI) schedules for food rewards. Rats were allowed food every 20, 40 or 60 seconds during tests. The temporal course of responding was obtained within the time interval (i.e., 20, 40 or 60 sec.) for a series of trials at each of the three fixed intervals. The results indicate: (a) adaptation effects over trials were dependent upon age with senescent rats have higher relative total response rates than young rats; and (b) senescent rats tended to make more responses in the initial portion of the fixed interval than young rats. These data show that temporal patterns of responding within an interval prior to a primary reward differ for young and senescent rats. The data also suggest that senescent rats have greater behavioral rigidity than young rats.

3. Food deprived young and senescent rats (male and female) were tested on 4-unit mazes to determine age or sex differences in learning and learning to learn ("learning sets"). The principle findings were that young animals learned more quickly than old animals, and that male rats learned more quickly than female rats. The major age differences occurred on the initial trial after shifting to a new problem suggesting that old rats had more difficulty than young rats in changing response patterns, once the response had been learned.

4. A complex maze was utilized to determine possible age differences in learning of specific culs-de-sac, and to obtain retention and extinction data. Senescent rats failed to learn the maze as quickly as young rats, and some failed to learn at all. Those rats which failed to reach criterion were allowed longer between trial intervals, but performance failed to

improve. When training trials were highly massed, 11 of 12 senescent slow-learners reached criterion by the third day of training. The previous failure of these senescent slow-learners to reduce errors was due primarily to perseverative, nonrandom types of errors based on tendencies to enter specific culs-de-sac on every trial. There were no differences in retention between old and young groups after 45 days. During extinction tests, senescent rats made fewer errors and ran faster to the goal box than young rats. It was concluded that age differences in learning and extinction were due to behavioral rigidity or relative lack of variability for senescent rats.

Proposed Course of Project: Further experiments have been designed to determine age differences in (a) bar pressing during long fixed ratio and fixed interval reward schedules, (b) performance and extinction as a function of effortfulness of response and (c) alternation behavior between two bars as a function of probability of reward. Additional maze studies will be included to: a) determine optimal between trial intervals for learning and retention, b) analyze the effects of maze complexity, and c) study the serial effects associated with learning of multiple maze problems.

In addition, some of the studies of behavioral rigidity will be repeated because the implications of the findings (especially those concerning massed training) are great. Furthermore, the studies relating behavior, aging and inheritance will be continued.

Honors and Awards: None

Publications:

Goodrick, C.: Learning and retention of a light contingent bar press response for three inbred strains of mice. J. Psychol., 67: 191-199, 1967.

Goodrick, C.: Operant responding of nondeprived young and senescent male albino rats. J. genet. Psychol., In Press.

Serial No. HD-AG26
1. Gerontology Research Center
2. Physiological Psychology
3. Baltimore, Maryland 21224

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Solution discrimination, preference and fluid ingestion of rodents as a function of age

Previous Serial Number: HD-AG26

Principal Investigator: C. L. Goodrick (20% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	.40
Professional:	.20
Others:	.20

Project Description:

Objectives: The effects of aging on sensation and perception are great. The purpose of this project is to determine some of the hereditary and environmental factors which mediate these effects.

Methods Employed: Our laboratory is especially concerned with an analysis of the chemical senses. In our work with gustation, we are using a technique which requires the animal to discriminate the more concentrated of two sucrose solutions. Other tests of taste preference (e.g., "salty", "sour" and "bitter") are also being developed. Tests of absolute preference thresholds are made by permitting the animal to choose between two bottles: one contains water while the other contains a given percentage of a substance for which the threshold is being determined. Differential preference thresholds are determined by comparing a fixed standard solution (e.g., 1 gm. sucrose/100 ml.) to a series of alternate comparison solutions (e.g., 3, 2, 1.5 gms. sucrose/100 ml.). In some of our studies we have used other methods such as single or multiple bottle tests. Water deprivation and ingestion during or over specific time intervals have also been employed. In all studies, however, animals have always been tested in their home cages so that novelty effects would not confound our findings.

Major Findings: 1. The sucrose absolute threshold has been studied for

young and senescent, food deprived and nondeprived rats (Total N=92). This study is a continuation of our previous work, however, the results are based on a longer period of testing and an improved technique for tube placement. The findings suggest that sucrose discrimination provides an excellent method of studying age differences in sensory discrimination (absolute or relative thresholds) and performance (total fluid ingested). Young rats discriminated the sucrose concentrations more precisely than senescent rats, but food deprivation had no effect upon this discrimination. Average ingestion rates of senescent rats were greater than rates for young rats, and rats on ad lib. feeding drank more than food deprived rats. Total ingestion rates increased more among food deprived than nondeprived rats, and young food deprived rats increased fluid intake proportionately more than senescent food deprived rats when the sucrose concentration was high. These last findings are consistent with earlier results from this laboratory.

2. Because of the implications of an earlier finding that discrimination is not related to food-deprivation, a study was done to determine if discrimination differences between young food-deprived and nondeprived rats (N=48) could be obtained as the sucrose concentration was increased. Standard sucrose concentrations of 0.0, 0.5, 1.0, 5.0, 9.0, and 18 grams sucrose/100 ml. were utilized. The findings were consistent: absolute and differential sucrose thresholds were not reliably different when food deprived and nondeprived groups were compared although total fluid ingested increased more for deprived than nondeprived groups. These findings underscore the importance of differentiating between performance and sensory discrimination.

3. Young and senescent inbred C57BL/6J mice were tested to determine age differences in fluid ingestion and sucrose discrimination. Senescent mice were found to ingest more tap water than young mice. However, during tests of sucrose discrimination, young mice increased fluid ingestion more than senescent mice and young mice ingested a higher proportion of sucrose solution than senescent mice for all concentrations. These groups were also tested at a later time for ingestion of a 10% ethyl alcohol solution. Young animals preferred the 10% alcohol solution, while senescent mice clearly preferred tap water in the two bottle tests. These data show that reliable behavioral data may be obtained from inbred strains of mice, and that age changes in sucrose discrimination and alcohol preference are similar in rats and mice.

Proposed Course of Project: Experiments will be designed to determine environmental and genetic determinants of fluid preferences as a function of age in rats and inbred mouse strains. For rats, analyses will be made of: 1) the effects of past experience upon preference behavior; 2) the effects of nutrition and drugs upon absolute and differential thresholds; and 3) isohedons (i.e., solutions which contain both sucrose and quinine over the entire range of preference values). Inbred strains of A/J and C57BL/6J mice and the F_1 and F_2 generations will be tested for absolute and differential thresholds over the entire age range.

Honors and Awards: None

Publications:

Goodrick, C.: Behavioral characteristics of young and senescent inbred female mice of the C57BL/6J strain. J. Geront., 22: 460-464, 1967.

Goodrick, C.: Taste discrimination and fluid ingestion of male albino rats as a function of age. J. genet. Psychol. In Press.

Serial No. HD-AG27
1. Gerontology Research Center
2. Physiological Psychology
3. Baltimore, Maryland 21224

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: An experimental analysis of rodent activity level and exploratory behavior as a function of age

Previous Serial Number: HD-AG27

Principal Investigator: C. L. Goodrick (20% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	.40
Professional:	.20
Others:	.20

Project Description:

Objectives: Activity level and exploration are two classes of behavior which are frequently studied in psychological research because each has been shown to be strongly and consistently related to drive and motivation. The objectives of this project are: 1. The development of reliable and valid tests of activity level and exploration, and the application of these tests to studies of aging and studies of inheritance; 2. the specification of variables which control such behavior.

Methods Employed: Studies include determination of the animal's normal home cage activities, patterns of sleep and wakefulness, wheel activity, free open field exploration and forced open field activity. Time sample methodology has been utilized in many experiments. Such research involves taking measures systematically during a particular time interval. In the present work several different test areas and time intervals have been used. For open field work, emotionality is normally measured by means of the defecatory reaction (i.e., no. of boli).

Major Findings: 1. In order to examine the relations of age and motivational level to activity and emotionality, young and senescent food deprived and nondeprived rats (N=92) were tested on a series of five trials in a circular plexiglas open field. Young rats remained at a higher average level of activity than senescent rats during all trials.

Although the rate of activity did not decline with trials for young rats, the mean activity scores of senescent rats did. Food deprived rats of both age groups were more active and less emotional than nondeprived groups. Emotionality of senescent rats remained at a higher level, while the emotionality of young rats declined. Senescent rats were consistently more emotional than young rats. These date supplement and replicate preceding research within this laboratory. They strongly suggest that activity and emotionality systems adapt over trials differently in young and old animals in the face of a persistence in the basic inverse relationship of activity and emotionality.

2. A study was completed to determine the feasibility of increasing basal wheel-running activity levels of rats by operantly conditioning them to increase their runway activity to obtain food. Young and senescent food deprived rats maintained within activity wheels to obtain basal activity levels and then they were trained to press a bar to obtain a food reward at the end of a runway opposite the bar. The senescent group increased their basal activity levels more than the young group during these training trials.

Proposed Course of Project: Further studies will determine age differences in exploration, locomotion and emotionality for male and female albino rats and for A/J and C57BL/6J inbred strains of mice. Operant responding with reward contingent upon general activity will also be utilized to attempt to change basal activity levels in wheels, open fields and in the home cage. Techniques for optimizing training methods to increase the behavioral responding of senescent rats and mice will be determined. Some parameters which we will vary are runway length, reward magnitude, and schedules of reinforcement during wheel running to obtain a food reward.

Honors and Awards: None

Publications: None

Serial No. HD-AG2(8)
1. Gerontology Research Center
2. Physiological Psychology
3. Baltimore, Maryland 21224

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Operant conditioning of heart rate in monkeys

Previous Serial Number: None

Principal Investigator: Bernard T. Engel (50% time)

Other Investigators: Theodore Weiss

Cooperating Units: None

Man Years

Total:	1.40
Professional:	.65
Other:	.75

Project Description:

Objectives: 1. To train monkeys to voluntarily control their cardiac rates. 2. To use this preparation to identify the physiological mechanisms associated with voluntary control of cardiac rate.

Methods Employed: 1. Procedures to date have been limited to the procurement of permanent equipment, to the design of specialized equipment and to the establishment of the research facilities. 2. Once the facilities become available and after we have established a reliable preparation, we plan on undertaking a series of studies to identify the physiological mechanisms underlying voluntary control of cardiac rate. Our techniques will include the use of pharmacological agents which facilitate or inhibit one or the other branch of the autonomic nervous system, and the use of stimulation, lesioning and direct recording techniques from various areas of the brain.

Major Findings: None

Proposed Course of Project: As outlined above.

Honors and Awards: None

Publications: None

Serial No. HD-AG11(8)(c)
1. Gerontology Research Center
2. Physiological Psychology
3. Baltimore, Maryland 21224

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Psychometric evaluation of pacemaker therapy in patients with complete heart block

Previous Serial Number: None

Principal Investigator: Theodore Weiss (20% time)

Other Investigators: Bernard T. Engel

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	.65
Professional:	.30
Other:	.35

Project Description:

Objectives: To assess the changes in intellectual and psychomotor performance in patients with complete heart block who have had pacemakers successfully implanted.

Methods Employed: 1. The introduction and increasing use of cardiac pacemakers in the past decade has revolutionized the treatment and prognosis of heart block. Dramatic reversals of block symptomatology are reported commonly, including striking improvement of mental status. However, no studies employing quantitative measures of psychological and psychomotor function have been reported to date. What seems to us another tantalizing question is whether less obvious, but measurable and significant psychological improvements could be demonstrated among pacemaker patients for whom dementia was not the indication for pacing.

2. One experimental group will consist of about 15 patients with established complete heart block in whom cardiac pacemakers are to be used. We also will include another group of about 5 patients for whom dementia is the pathological indication for pacing.

We will use two control groups. The first will be composed of about 15 patients who are scheduled for relatively minor surgical procedures. These patients would be matched for age, sex, race, education and

income with our first group of experimental subjects.

The second control group will be about 15 patients undergoing mitral valvulotomy.

We plan to test our subjects twice, shortly before surgery - one or two days whenever feasible - and one or two weeks post operation. We will employ several tests.

1. Wechsler Adult Intelligence Scale (WAIS).
2. Reitan Trail Making Test.
3. Reitan Finger Tapping Test.
4. Reaction Time - Simple and Disjunctive.
5. Hand Dynamometry.

In addition to these tests, neurological examination of all the patients and informal interviews of all the pacemaker patients will be performed.

Major Findings: None

Proposed Course of Project: As outlined above.

Honors and Awards: None

Publications: None

Serial No. HD-AG14(8)(c)
1. Gerontology Research Center
2. Physiological Psychology
3. Baltimore, Maryland 21224

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Relationships between cardio-respiratory activity, behavior and aging

Previous Serial Number: None

Principal Investigator: Bernard T. Engel (15% time)

Other Investigators: Phillip R. Thorne

Cooperating Units: None

Man Years

Total:	.90
Professional:	.25
Other:	.65

Project Description:

Objectives: The purposes of this program are:
1. to study the interaction between cardiac cycle phase and respiratory cycle phase in different aged groups; 2. to study the relationship between reaction time and cardio-respiratory phases among different aged groups.

Methods Employed: Previous research suggests that reaction time is different during inspiration and expiration and during the QRS, T and P wave phases of the cardiac cycle. However, all these studies have introduced reaction stimuli at various times and then retrospectively determined the cardio-respiratory phases in which the stimuli fell. These procedures are inefficient, and the results are open to question since the subject is required to respond too often and usually becomes fatigued and even bored. A much more efficient procedure would be one based on techniques which can detect the various cardio-respiratory phases and can automatically introduce stimuli at appropriate times and these phases. Our project is designed to create these techniques.

Since it is relatively simple to detect R waves and to predict the occurrence of the T wave in the normal ECG, the only technical problem is P wave detection. Furthermore, since it is a relatively simple matter to detect inspiration and expiration, this study reduces to a development of

procedures for detecting the P wave. Electronic devices are being developed which will predict the occurrence of P waves on the basis of information about previous cardiac cycles. Once the equipment has been developed, it will be tested in normal human subjects in two conditions: 1) at rest; and 2) quiet but alert. Ultimately, the system will be tested on subjects during reaction time tasks.

Major Findings: 1. Our first attempt at p-wave detection was to design a system which would predict the occurrence of a p-wave on the basis of its occurrence during the previous cardiac cycle. The following will illustrate our method:

1. Assume an R-R interval of duration, T;
2. Assume the p-wave occurs at some fixed interval, I, within the cardiac cycle.
3. Then the proportion of the cardiac cycle when the p-wave occurs will be I/T or K.
4. If K is constant, irrespective of rate, then once one has determined K for a given individual he should be able to predict the occurrence of P: 1) if he knows the duration of the preceding R-R interval; and 2) if the contemporary R-R interval is approximately of the same duration as the preceding one.

5. Our system made this analysis and prediction on-line, on a beat-to-beat basis.

2. Our results with four subjects indicated that: 1) the overall accuracy of the system ranged from about 60 to 95 percent correct predictions; 2) prediction was about 10 to 15 percent better in the awake or alert subject than in the resting or drowsy one; 3) prediction was somewhat better when the subject was inhaling than when he was exhaling; 4) accuracy tended to vary inversely with the variability of beat-to-beat heart period - if the variability was great, predictability tended to be relatively poor.

Proposed Course of Project: 1. Analyses of the preceding data suggest that the major source of error in our present system is the cycle to cycle change in cardiac period. Our assumption of small beat-to-beat change is wrong in some persons. Since the major source of variation in cardiac period under the conditions of our study is respiratory sinus arrhythmia, we intend to modify our electronic system to include not only information about the preceding R-R interval but also information about the state of the respiratory cycle.

2. Once this system has been developed and is operating successfully, it will enable us to move to our primary research goal: a study of the effect of age on the relationship between phases of the cardiac and respiratory cycles and reaction time.

Honors and Awards: None

Publications: None

Serial No. HD-AG16(8)(c)
1. Gerontology Research Center
2. Physiological Psychology
3. Baltimore, Maryland 21224

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Modification of cardiac arrhythmias through voluntary control

Previous Serial Number: None

Principal Investigator: Theodore Weiss (50% time)

Other Investigators: Bernard T. Engel

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	1.05
Professional:	.75
Others:	.30

Project Description:

Objectives: 1. To determine whether persons with cardiac arrhythmias can learn to control and modify their cardiac rhythms, and can maintain such changes.

2. To determine whether patients with cardiac arrhythmias can learn to control and modify their cardiac rhythms sufficiently to produce significant clinical improvement.

Methods Employed: 1. Work in my laboratory (prior to my coming to the GRC) has shown that normal, healthy men can be taught to slow or speed their heart rates (Engel, B. T. and Hansen, S. P.: Operant conditioning of heart rate slowing. Psychophysiol., 3: 176-187, 1966. Engel, B. T. and Chism, R.: Operant conditioning of heart rate speeding. Psychophysiol., 3: 418-426, 1967.). We are currently preparing a paper which will report that patients with cardiac arrhythmias can learn to control not only their heart rates but also their heart rhythms (Engel and Melmon, in preparation). This study will also report that the changes in cardiac function seen in these patients are sufficiently great, and endure sufficiently long to suggest that the techniques we were using might be therapeutic.

2. Since the goals of this research are: a) to confirm the previous work; and b) to establish the clinical significance of the method, all subjects will be seen in the laboratory and will be trained as described

in the references cited above. In addition, subjects will also be retested at periodic intervals for several years to enable us to learn more about the endurance of the procedures.

3. Clinical improvement will be judged from several indices:
   a. Subjective reports of general feeling state, including such things as exercise tolerance, dizziness, "skipped beats", etc. Since the exact nature of the reports vary with the arrhythmia, it is not possible to be more specific here.
   b. Electrocardiographic signs at rest and during exercise.
   c. Other signs of cardiac function based upon physical diagnostic studies.

Major Findings: There have been no findings. All our time has been spent in procuring equipment and developing the laboratory.

Proposed Course of Project: After our equipment has arrived and been put into operation, we will admit outpatients from the BCH Cardiac Clinic into the Gerontology Research Ward where they will be kept for the period of study (about 3 weeks).

Honors and Awards: None

Publications: None

Serial No. HD-AG28
1. Gerontology Research Center
2. Molecular Biology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Structure of Nucleic Acids

Previous Serial Number: HD-AG28

Principal Investigators: G. Eichhorn (80% time)
Y. Shin (100% time)
H. von Hahn (100% time) (Left 12/31/67)
J. Butzow (100% time) (E.O.D. 9/7/67)
J. Rifkind (50% time) (E.O.D. 1/1/68)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	5.89
Professional:	3.55
Others:	2.34

Project Description:

Objectives: (1) To study the reactions of metal ions, proteins and synthetic polypeptides with nucleic acids. (2) To find specific reactions of the individual nucleotides. (3) To understand the biological significance of metal ions in nucleic acid function. (4) To develop methods for the determination of nucleotide sequence in nucleic acids.

Methods Employed: Conditions are determined for the binding of metal ions to (1) the phosphates on polynucleotide chains and (2) the bases attached to the ribose phosphate backbone. Metal binding to phosphate can be used to degrade the polymers, and the degradation reactions are studied for possible specificities in their attack on different internucleotide bonds. Metal binding to the bases competes with the hydrogen bonding of these bases and thus affects the helix-coil transitions of the polymers. Protein binding to the phosphates simulates a condition of high electrolyte concentration. Through studies using a combination of protein and a metal which binds to the bases it is hoped to find clues to the participation of metal ions in biological phenomena such as DNA replication and protein synthesis. Preferential binding of metal ions to some of the bases may be used in sequential analysis either by rendering the affected bases more susceptible to hydrolytic attack or by specifically inhibiting the attack of nuclease enzymes upon the metal bound nucleotides.

Major Findings: A. The Binding of Metals and the Unwinding and Rewinding of DNA. We have previously shown that divalent metal ions can be placed in the following order of ratio of base binding ability to phosphate binding ability: Mg< Co, Ni< Mn< Zn< Cd< Cu. We had also demonstrated that Cu(II) and Zn(II) can bring about a reversible unwinding and rewinding of DNA. Using DNA melting experiments we have now shown that metal ions generally can function in the catalysis of this helix-coil transition, and that the extent of rewinding possible, after unwinding, is determined by the position of the metal in the above series. Thus Mg(II) produces no rewinding, Co(II), Ni(II), and Mn(II) a gradually increasing amount of rewinding, and Zn(II) complete rewinding on cooling a heat-denatured solution. Cd(II) and Cu(II) give no rewinding at all on cooling, but the addition of electrolyte brings instantaneous rewinding with Cd(II) and rewinding after standing with Cu(II). The metals in the series up to zinc bind strongly enough to bases to hold them in register in the coiled form and yet strongly enough to phosphate to reform double helix at low temperature. Cd(II) and Cu(II) bind too strongly to the bases to be displaced by cooling, but they can be displaced by electrolyte, Cu(II) most readily.

Spectrophotometric studies reveal that metal ions displace the absorption maximum of DNA, following the above sequence, displacements increasing from left to right. The sequence is thus borne out by three phenomena: effect on T_m of DNA (last year), effect on unwinding-rewinding, and effect on DNA absorbance.

Fe(II) behaves like Cd(II) and Cu(II), but no rewinding can be effected by electrolyte addition.

The reversible reaction with zinc has been further elucidated. In one experiment zinc was added at elevated temperature to DNA denatured in the absence of zinc. The addition of zinc at this point did not produce rewinding, demonstrating that zinc must be present in the unwinding step, forming crosslinks, in order to produce rewinding on cooling. The identity of the native DNA and zinc-renatured DNA was further proved by the finding that both the sedimentation coefficient and the peak in a CsCl density gradient are unchanged before and after DNA and zinc have been heated and cooled. The effect of zinc on melting curves of poly dAT (DNA containing no guanine and cytosine) indicates no base binding, thus implicating zinc binding to the GC pairs. The lack of displacement of the absorption maximum of dAT (but not DNA) by zinc corroborates this observation.

The previously demonstrated unwinding of DNA by ATP was explained by the tetranegative charge on the ATP. EDTA, which has the same charge, produces the same effect. Other metal complexing agents, e.g. glycine and 8-hydroxyquinoline have no such unwinding effect. Thus the effect of ATP is probably due to its charge, and not to its ability to bind metals, of some importance in the replication and transcription reactions that require nucleoside triphosphates. UTP is somewhat less effective than ATP.

B. Depolymerization of Polynucleotides by Zinc. We have already reported that the cleavage of the phosphodiester bonds of polynucleotides by zinc involves a specificity of potential importance in sequence determination. Cleavage adjacent to guanine occurs least readily and cleavage adjacent to uracil most readily. Though much effort has been expended in the variation of reaction conditions to increase this specificity, we have had only minor success. Changes in temperature, metal concentration, pH, and other variables, have not greatly modified the points of cleavage. We have therefore decided to learn more about the mechanism of this degradation reaction, and hope that our goal can be obtained more rationally, instead of empirically.

The degradation to soluble oligonucleotides has been shown to be complete in 2 hrs. at 64°. If phosphate is split from the ends of the oligonucleotides, the assay for orthophosphate shows that only 75% of the total possible amount is produced after 5 days, after which no more reaction occurs. It is thus evident that the polynucleotides are only incompletely degraded. This is borne out by examination of the end products (with terminal phosphate removed) of the degradation of poly A. Only 71% monomer is produced, along with 27% dimer and 2% trimer (and no detectable higher oligomers). Mathematical analysis of these observations leads to 85% reaction completion instead of the 75% observed. The discrepancy may be explained by the identification of a cyclic phosphate during the degradation of poly C. Any cyclic phosphate formed would not be detected in our determination of points of cleavage. The formation of cyclic phosphates suggests that these substances may be intermediates in the phosphodiester bond cleavage. Metal ions binding to phosphate would make the phosphate less negative and thus attract the 2' OH group to it, producing the unstable cyclic phosphate, which would decompose and produce the cleavage.

DNA, unlike RNA, is then not cleaved by zinc, since it has no 2' OH group that can form the cyclic intermediate. This finding is a very useful one for separating RNA from DNA by degrading the former, leaving the latter intact.

To the extent that the degradation is specific, the 3' and 5' sides of the phosphodiester bond appear equally vulnerable. This conclusion results from the fact that the identifiable cleavage sites are the same, whether the oligonucleotides are degraded by 3' specific alkali or 5' specific venom phosphodiesterase.

C. The Reaction of Polynucleotides with Polypeptides. Because of the assumed importance of protein (histone) binding to DNA, we are conducting systematic investigations into the nature of the bonding between the two types of biomacromolecules. We have found that polylysine shifts the absorption maximum of poly A, and poly A shifts the absorbance of polylysine. Spectrophotometric titration of polylysine with oligo A indicates that at a certain concentration of oligo A the polylysine undergoes a conformational change. Such a change in conformation could be important in the release of protein from nucleic acid molecules that plays a role in cell differentiation

theory.

D. The Effect of Metal Ions on the Preparation of Histones. It had previously been indicated (by Dr. von Hahn in Switzerland) that a histone fraction from calf thymus appears unusually strongly bound to DNA and that the quantity of this fraction increases with age. We have now found that rat liver nucleohistone does not contain such a histone fraction, but does contain four histone fractions with different amino acid compositions that are separated as four peaks on Sephadex. The yield of histones is determined to a large extent by the amount and nature of metal ion solutions present during the preparation; the effectiveness of metal ions in histone isolation falls in the order Cd>Mn>Mg>Na. The divalent ions studied are in the sequence of decreasing phosphate to base affinity, as discussed in Section A. The addition of EDTA gives considerably lower yield of histones, presumably by complexing indigenous metal ions.

Of even more interest than the effect of the metal ions on the yield of total histone is their effect on the relative amounts of the various fractions produced. The absence of divalent ions almost totally eliminates two of the peaks. All four peaks are characterized not only by their elution position, but also by Lowry (protein) and Sakaguchi (arginine) determinations, absorbance, and, in some instances, total amino acid analysis. Generally a given peak gives similar values for all of these determinations, regardless of the nature of the preparation. When two of the peaks are eliminated in the absence of the metal ions, fractions of residual protein taken at the elution positions for the peaks give different analytical data. The metal-sensitive peaks are histones rich in arginine, indicating that the poly-arginine structure may be strongly affected by metal ions.

E. Age and Species Differences in Histone Fractionation. Histone fractions were obtained as above from livers of rats of different ages. In the presence of divalent metals, "young" rats (up to 12 months) always exhibit all 4 peaks. In "old" rat (26 months or more) preparations the same two peaks that are always eliminated in the absence of divalent metals were not observed in about half the cases studied. Thus the fractionation pattern of these "old" rats in the presence of divalent metals resembles the pattern from young rats in the absence of the metals, in some but not all instances.

The apparent implication of this finding is that at least one major reason for observed age differences in DNA-histone binding lies in the change in the native ionic environment of the nucleohistone that may occur with age. Since added metal ions have a profound effect on histone fractionation patterns, it follows that the ions initially present can have a similar effect.

We have some evidence that the different fractionation patterns of histones from calf thymus and rat liver may also be partly due to differences in ionic environment.

F. The Effect of Metal Ions on RNA Synthesis. Metal ions are essential

components of the transcription of mRNA on DNA catalyzed by the RNA polymerase enzymes. We have studied the effects of various metal ions on enzymes isolated from E. coli as well as purchased materials. Present indications are that in the presence of Mg(II), which is generally used as an activator, Mn(II) enhances RNA synthesis, while Cu(II) and Zn(II) diminish it. These studies are complicated by the presence of numerous agents that are generally deemed necessary for these enzymatic reactions but that react with metal ions. We are attempting to remove these substances from the reaction so that the effects of the metal ions can be isolated.

Proposed Course of Project: We are continuing the studies on the effects of metal ions on RNA polymerase and hope also to study the replication reaction catalyzed by DNA polymerase. We hope to find some clues to unwinding and rewinding of DNA in transcription and replication from these studies as well as from further work on the effect of nucleoside triphosphates on DNA. We are on the way toward a determination of the mechanism of metal-induced RNA degradation by the determination of products from small oligonucleotides as well as polynucleotides. The binding of polynucleotides and proteins, and its mediation by metals, will be further investigated by U.V. and I.R. spectrophotometry as well as by nuclear magnetic resonance. We are also continuing to obtain data on the effect of metals on nucleohistone fractionation and the implications of this phenomenon on the age differences of nucleohistones.

Publications:

1. Shin, Y. A. and Eichhorn, G. L.: The interaction of metal ions with polynucleotides and related compounds. XI. The reversible unwinding and rewinding of deoxyribonucleic acid by zinc(II) ions through temperature manipulation. Biochemistry 7: 1026-1032, 1968.

2. Butzow, J. J. and Eichhorn, G. L.: Physical chemical studies on the age changes in rat tail tendon collagen. Biochim. Biophys. Acta 154: 208-219, 1968. (This publication is from a previously completed project).

3. von Hahn, Holger P.: Failures of Regulation Mechanisms as Causes of Cellular Aging. In B. L. Strehler (Editor): Advances in Gerontological Research, Volume 3, in press.

Honors and Awards: None

Serial No. HD-AG30
1. Gerontology Research Center
2. Molecular Biology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Function of Metal Ions in Enzymatic Reactions

Previous Serial Number: HD-AG30

Principal Investigators: G. Eichhorn (20% time)
P. Clark (100% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	1.66
Professional:	1.2
Others:	0.46

Project Description:

Objectives: To arrive at an understanding of the mechanisms by which metal ions participate in enzymatic reactions, and also to determine why different metal ions are required for different enzymatic processes.

Methods Employed: It is assumed that in any metal-catalyzed enzymatic process the metal ion must serve either (1) as an active site, by direct attachment to the substrate, or (2) it must react with the enzyme protein, and thus alter the secondary and tertiary structure of the latter, producing the active configuration. It is postulated that, whenever scheme (1) applies, the metal should be capable of bringing about a non-enzymatic reaction; failure of the non-enzymatic reaction, inversely, would favor scheme (2).

Specifically selected enzymatic substrates are treated with metal ions under varying conditions, and the nature of the metal-substrate interactions detected by physical measurements, e.g., spectrophotometry. The interaction of the metals with the enzyme protein is studied by macromolecular techniques, such as ultracentrifugation.

Major Findings: A. Ribonuclease. We have previously studied the activation and inhibition of crystallized bovine pancreatic ribonuclease by metal ions. We have now carried out the same studies with ribonuclease A, which is a chromatographically obtained fraction of the crystallized enzyme whose structure has now been partly worked out crystallographically. The metal ion effects on ribonuclease A are identical with those

previously observed for the crystallized mixture. The similarity of the other components of this mixture to ribonuclease A, deduced from other experiments, is thus also apparent from the metal interactions.

We have been able to correlate the effect of the metals on enzyme activity with the activity of the enzyme (RNAse A) to bind metal ions. Evidence indicates that the latter are bound to the terminal lysine and to the four histidines, two of which are on the active site and two are not on this site. Our metal interaction data point to one type of activating site and one type of inhibiting site. We assume that the terminal lysine-bound metal has no effect on enzyme activity, since this lysine can be removed or modified in many ways without influencing the activity. Thus the two histidines on the active site constitute one type of metal binding group and the other histidines constitute another. We do not know which is activated and which is inhibited.

B. Deoxyribonuclease. We have been engaged in an effort to modify the specificity of DNAse by metal interaction, so that the metal enzyme complex will be useful in studies of nucleotide sequence in DNA. The essential features of the procedure have been previously discussed. These have been modified in many details to give greater reproducibility of results and higher specificity. Qualitatively, our results are still as reported last year; i.e., in the presence of Cu(II) thymine bonds are preferentially cleaved, at the expense of guanine bonds. Quantitatively, these results are now reproducible within 3%, and the specificity is better than in the zinc degradation of RNA: only 1/3 of the guanine-linked phosphodiester linkages are cleaved.

Proposed Course of Project: This project is of course closely linked with that on Structure of Nucleic Acids. We hope to be able to use our findings in the sequence determination of a small oligodeoxynucleate.

Honors:

1. Gunther Eichhorn was invited to present one of the five plenary lectures at a symposium on homogeneous catalysis at the 11th International Conference on Coordination Chemistry. He was also asked to be on the discussion panel of the symposium.

2. Gunther Eichhorn was asked to be on the Editorial Board of a Journal of Coordination Chemistry when this journal is established.

Publications:

1. Eichhorn, G. L.: Macromolecular Coordination Compounds in Biological Systems. In A. E. Martell (Editor): Coordination Chemistry, in press.

Serial No. HD-AG17(8)(c)
1. Gerontology Research Center
2. Molecular Biology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Relation of Structure to Function in Hemoglobin

Previous Serial Number: None

Principal Investigators: J. Rifkind (50% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	.25
Professional:	.25
Others:	None

Project Description:

Objectives: (1) To study the binding of ligands to hemoglobin. (2) To determine the role of the protein in controlling the binding of ligands to hemoglobin. (3) To study variations in the extent of protein involvement and to understand the biological significance of these variations.

Methods Employed: Binding experiments are performed with a ligand like oxygen. These experiments are performed by a method where the oxygen bound is measured directly and by a spectrophotometric method. Different preparations of hemoglobin are studied under varying conditions. From the binding curves and the differences between the direct binding curve and the spectrophotometric binding curve, information is obtained regarding the mechanism for the binding and the role of the protein.

Major Findings: In the past year before coming to NIH, Joseph Rifkind was working on this project at the University of Minnesota. The major finding of this project was to establish that direct binding experiments and spectral binding experiments measure two different aspects of the binding process. This was demonstrated to be true at least of horse hemoglobin, under certain conditions. In order to accomplish this a new technique was developed for doing direct binding experiments. These findings are particularly significant because most workers in this area do binding experiments spectrophotometrically and assume they are meaasuring the oxygen bound.

Proposed Course of Project: (1) We want to obtain detailed enough binding

data on horse hemoglobin by both methods in order to quantitatively interpret the differences in terms of the changes taking place in the protein as the oxygen is bound. (2) We want to look at human hemoglobin and compare the results on human and horse hemoglobin. There is reason to believe that the interaction between the hemes and the protein subunits are quite different for horse and human hemoglobins. If this is true, it will show up in our experiments. (3) The horse hemoglobin which we have been working with contains two major components. These two components have different binding characteristics and the relative amounts of these two components is thought to depend on the age of the red cell. It is planned to separate these two components (this has been done), try to determine exactly what chemical differences there are between both of them (it is thought that there is an amino acid that gets oxidized), and study the binding characteristics of both components by the spectral and direct methods. (4) It is possible that the age difference in the binding of oxygen to hemoglobin may be related to a more rapid breakdown of one component of hemoglobin into another component. We, therefore, plan to investigate the variation of the various components of human hemoglobin as a function of age.

Publications: None

Honors and Awards: None

Serial No. HD-AG18(8)

1. Gerontology Research Center
2. Intermediary Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Effect of Age on the Mechanism of High-Energy Transduction in Brain Mitochondria

Previous Serial Number: None

Principal Investigators: Bertram Sacktor (15% time) (EOD 10/16/67)
Joel Garbus (100% time) (EOD 11/12/67)

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.7
Professional:	1.0
Others:	0.7

Project Description:

Objectives: Increased lability of the oxidation-phosphorylation system in mitochondria from brains of aged animals is well documented (Weinbach and Garbus, J. Biol. Chem. 234(2), 1959). An additional, and perhaps more significant, factor in the decrease in maximal ATP availability during senescence may stem from changes in the mechanism of high energy transduction, i.e., the transfer of chemical energy from ATP, synthesized within the mitochondria, to extramitochondrial acceptors, which are the immediate energy donors for neural function. The localization of three binding sites for hexokinase activity in brain, two, mitochondrial, and one, extramitochondrial, suggests an unique and critical mechanism for energy transduction in brain. This study aims to explore this novel transducing system and to test the hypothesis that this system is modified during biological aging with consequences on maximal neural function and performance.

Methods Employed: Procedures were developed for the isolation of relatively pure mitochondrial fractions from rat brain and for the determination of functional activity in these fractions.

Major Findings: A simple procedure utilizing rapid differential centrifugation was developed for the isolation of functionally intact brain mitochondria. Comparing mitochondria from the brains of 11 and 27 months old rats, it was found that there was a significant decline in respiratory

control (regulation by demand) of oxidative phosphorylation in the older animals. This decline was observed with two substrates, succinate and α-ketoglutarate, but not with pyruvate or glutamate. Serum albumin improved the respiratory control with the mitochondria from old animals, suggesting that these organelles are more susceptible to changes in environment. There were no age-related changes in the respiratory rate or efficiency of oxidative phosphorylation (P/O ratio) between the two groups.

Proposed Course of Project: The effect of age on the mechanism of high-energy transduction in brain mitochondria will be continued.

Honors and Awards: None

Publications: None

Serial No. HD-AG19(8)

1. Gerontology Research Center
2. Intermediary Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Mechanism of Action of Thyroid Hormone in Regulating Intermediary Metabolism and Homeostasis.

Previous Serial Number: None

Principal Investigators: Bertram Sacktor (15% time) (EOD 10/16/67)
Bernard Bulos (100% time) (EOD 3/10/68)

Other Investigators: Gilbert Isaacs

Cooperating Units: Medical Research Laboratory, Edgewood Arsenal, Maryland

Man Years

Total:	0.7
Professional:	0.5
Others:	0.2

Project Description:

Objectives: Effective understanding of age-related alterations in the function of thyroid hormone in regulating and controlling intermediary metabolism and homeostasis is largely dependent on knowledge of the mode of action of the hormone at the cellular and enzymatic levels. This investigation aims to provide information on the mechanism of the thyroid-induced specific synthesis of the mitochondrial α-glycerophosphate dehydrogenase and on the underlying basis for differences in response of peripheral tissues to the hormone. A cognate objective is to answer the fundamental question as to the site of synthesis of a mitochondrial enzyme and how this protein becomes incorporated into the mitochondrial cristae *in vivo*.

Methods Employed: Enzymatic and ultra-structural techniques were employed to determine the mechanism and the site of the thyroid-induced synthesis of mitochondrial α-glycerophosphate in rats. The perfused heart is used to examine thyroid effects at a more physiological level.

Major Findings: Thyroid hormone was found to induce the specific synthesis of the mitochondrial α-glycerophosphate dehydrogenase. The activity in heart and liver for euthyroid, hyperthyroid, and hypothyroid rats are, respectively, 13.6, 41.9 and 2.7, and 7.9, 83.1 and 2.0 µmoles dihydroxyacetone phosphate formed x min^{-1} x g^{-1} mitochondrial protein.

Perfused hearts from euthyroid rats have a rate of lactate utilization half that of pyruvate utilization. The findings that neither lactic dehydrogenase activity nor lactate permeability is limiting, suggested that the availability of oxidized nicotinamide adenine dinucleotide (NAD or DPN) may regulate the oxidation of lactate. The availability of NAD for the cardiac tissue may, in turn, be controlled by the α-glycerophosphate shuttle system, discovered earlier in insect flight muscle. The flux of the α-glycerophosphate cycle can be enhanced by an increase in the synthesis of the mitochondrial α-glycerophosphate dehydrogenase, which is induced by thyroid hormone. To test this hypothesis, the utilization of lactic, pyruvate and glucose were measured in hyperthyroid, hypothyroid as well as euthyroid rats. It was found that the rate of lactate utilization by hearts of hyperthyroid animals is increased 2-fold, now equalling the rate of pyruvate utilization. The thyroid state of the animal had no significant effects on the rates of pyruvate or glucose oxidation or on the redox state of the pyridine nucleotide-linked systems, assuming uniform distribution of the redox partners in the heart. These findings demonstrate, for the first time, a specific enzymatic target and concomitant physiological effect of thyroid hormone.

Proposed Course of Project: The mechanism of the thyroid induced specific synthesis of the mitochondrial α-glycerophosphate dehydrogenase and the basis for differences in response of peripheral tissues to the hormone will be sought. Rats of varying age will be used to determine age-related alterations in thyroid hormone function.

Honors and Awards: None

Publications: None

Serial No. HD-AG20(8)

1. Gerontology Research Center
2. Intermediary Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Mechanism of the Decrease in Maximal Work Performance of Flight Muscle during the Senescence of Insects.

Previous Serial Number: None

Principal Investigators: Bertram Sacktor (20% time) (EOD 10/16/67)
Charles Childress (100% time) (EOD 10/16/67)

Other Investigators: Ernest Bueding

Cooperating Units: Johns Hopkins University, Department of Pathobiology, Baltimore, Maryland

Man Years

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: Studies of work performance, muscular function, in insects show that the duration of flight and wing beat number and frequency decrease markedly during aging. Maximal flight ability in flies is dependent on the carbohydrate energy reserves, i.e., glycogen and trehalose. Evidence suggests that the synthesis, mobilization and utilization of these carbohydrates are tightly regulated and controlled. This investigation seeks to elucidate the mechanism of the control processes and to determine how senescence modifies these regulatory systems.

Methods Employed: The regulatory mechanisms controlling carbohydrate utilization at the phosphorylase site was determined.

Major Findings: Phosphorylases a and b have been purified from blowfly flight muscle by a procedure involving acid precipitation, $(NH_4)_2SO_4$ fractionation, heat treatment at alkaline pH, and finally column chromatography on DEAE cellulose using pH and ionic strength gradient elution. The final products have specific activities of 12 to 15 units/mg protein when assayed with KOH extracted glycogen at 30°C. This activity is comparable to that of crystalline rabbit skeletal muscle phosphorylase b, however, analytical ultracentrifugation revealed the presence of at least two protein components. Phosphorylase kinase has also been partially purified and is capable of

converting phosphorylase b from either flight muscle or rabbit skeletal muscle to the a form and requires ATP for this conversion. Kinetic measurements on phosphorylase a reveal that the levels of each of the two substrates, glycogen and Pi, and of the activator AMP, affect the apparent kinetic constants for the other two. As the glycogen concentration is varied from .17 mM to 1.7 mM end groups, the apparent values for Km for AMP and Pi range from 9×10^{-7}M to 4×10^{-7}M and 24 mM to 10 mM, respectively. As the Pi concentration is varied from 16 mM to 80 mM, the Km values for AMP and glycogen range from 7×10^{-7}M to 4×10^{-7}M and .75 mM to .28 mM, respectively. As the level of AMP is varied from 3×10^{-7}M to 2×10^{-6}M, the Km values for Pi and glycogen range from .130 M to .016 M and 1.0 mM to 0.62 mM, respectively. The most dramatic effect is that of AMP upon the Km for Pi. Under the conditions which prevail in vivo the Km values for AMP, glycogen, and Pi are 7×10^{-7}M, 0.83 mM, and 11.0 mM, respectively.

Similar patterns of substrate and modifier interaction are seen with phosphorylase b. The main differences are as follows:

(1) The apparent Km for AMP is 100-fold higher, approximately 2×10^{-4}M, also depending upon the levels of substrates.

(2) Phosphorylase b is strongly inhibited by ATP ($K_i \approx 7$ mM) at the levels observed in vivo whereas phosphorylase a is not inhibited. Inhibition is competetive with AMP.

(3) Preliminary results indicate that the Km for Pi may be 2-4 fold higher (20-40 mM) than for phosphorylase a under conditions of saturation with glycogen and AMP.

Under simulated in vivo conditions phosphorylase a appears to have approximately 50% of its potential activity were all substrates saturating, whereas phosphorylase b seems to have approximately 5% or less of its potential activity. With all substrates saturating (also AMP) and no inhibitors present the potential activities of a and b are equal. In the presence of 15 mM ATP and 14 mM Pi, increasing the level of AMP from .2 mM to .6 mM, as occurs on initiation of flight, nearly doubles the activity of phosphorylase b, however, the maximal rate is only 10% of the potential activity which is probably too low to account for the rate of glycogenolysis seen in vivo.

Phosphorylase a is stimulated 2-3 fold by AMP whereas phosphorylase b is inactive without AMP.

Values for Km are as much as 5-fold higher for native glycogen than for KOH treated glycogen whereas values for Vmax are 50% of those found for KOH glycogen.

Proposed Course of Project: The regulatory mechanisms controlling carbohydrate utilization at the phosphorylase and trehalase sites will be studied further. These systems will be examined in different aged flies because older flies show marked decreases in maximal work performance, which is dependent on carbohydrate metabolism and its regulation.

Honors and Awards:

Dr. Sacktor presented seminars on Regulatory Mechanisms in Insect Flight Muscle, In Vivo, to:

1. Department of Biology and Biochemistry, University of Southern California, Los Angeles, California
2. Department of Physiology, University of California, Berkeley, California
3. Department of Biochemistry, Temple University, and Fels Research Institute, Philadelphia, Penna.
4. NICHD, NIH, Bethesda, Maryland

Publications: None

Serial No. HD-AG23(8)
1. Gerontology Research Center
2. Intermediary Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Investigations on Inter- and Intracellular Transport Mechanisms

Previous Serial Number: None

Principal Investigator: Bertram Sacktor (50% time) (EOD 10/16/67)

Other Investigators: I. William Grossman

Cooperating Units: Department of Pathology, Sinai Hospital, Baltimore, Md.

Man Years

Total:	2.75
Professional:	.50
Others:	2.25

Project Description:

Objectives: Previous physiological studies by others on carbohydrate metabolism and renal function suggest marked age-dependent changes in the biochemical properties of cellular and subcellular membranes that alter the transport of glucose and ions into cells and between intracellular compartments. The paucity of information on the enzymatic mechanisms for the transport of these metabolites frequently limited interpretation of the experimental observations. An investigation of the enzymatic pathways mediating the active transport of glucose and ions was initiated to provide this needed information.

Methods Employed: Modern biochemical procedures including enzyme kinetics and purification, column and paper chromatography, radioisotopes, subcellular fractionation, histochemistry and electron microscopy are used.

Major Findings: A novel mechanism for the active transport of glucose has been proposed. The clue to this mechanism came from our finding that the enzyme trehalase, which hydrolyzes the disaccharide trehalose to its two glucose moieties and was reported previously only from microorganisms and insects, is exceptionally active in mammalian kidney and small intestine. The enzyme is absent from other tissues which are not capable of active transport of sugar, in contrast to the kidney and small intestine. The enzyme has now been found in 11 species, including man. The rabbit has the highest activity, 17 μmoles of glucose x min^{-1} x g^{-1} wet wt. Trehalase

is localized in the renal cortex and absent from the medulla and papilla. A histochemical method for trehalase activity was developed and employed to demonstrate that the disaccharidase is specifically localized in the renal cortical tubules, the site of glucose resorption in the intact animal. Subcellular fractionation of the cortex shows that the enzyme is bound to the membranes, cosedimenting with hexokinase, alkaline phosphatase, and 5'-nucleotidase, the latter an enzyme marker for plasma membrane. Kidney trehalase has been purified 50-fold by solubilization with deoxycholate and fractionation by heat, ethanol and DEAE chromatography. The enzyme is specific for trehalose, other α-glucosides and oligosaccharides are inactive with the exception of maltose. Crude preparations of kidney hydrolyze maltose at a rate 10% that of trehalose, but the two activities are separable during purification of trehalase. Kidney trehalase has a Km of 3.5mM. The pH optimum is 6.3.

Also found in the renal cortex are the enzymes which catalyze the following reactions:

(1) Glucose + ATP $\longrightarrow$ Glucose-6-P + ADP (Hexokinase);
(2) Glucose-6-P $\longrightarrow$ Glucose-1-P (Phosphoglucomutase);
(3) UTP + Glucose-1-P $\longrightarrow$ UDPGlucose + PP (Uridine pyrophosphorylase);
(4) UDPG + G-6-P $\longrightarrow$ Trehalose-6-P + UDP (Trehalose-6-P synthetase);
(5) Trehalose-6-P $\longrightarrow$ Trehalose + P (Trehalose-6-P phosphatase);

in addition to:

(6) Trehalose $\longrightarrow$ 2 Glucose (Trehalase).

The overall scheme is

2 Glucose + 2ATP + UTP $\longrightarrow$ 2 Glucose + 2ADP + UDP + PP + P.

Trehalose-6-P phosphatase, part of hexokinase, in addition to trehalase, are associated with the membranes. The enzymatic rate of each of the enzymes is sufficient to account for the rate of renal resorption in the intact animal. The formation of UDPG, reaction (3), is inhibited by phlorhizin, 50% by 2×10^{-4}M.

Trehalose-6-P phosphatase has a pH optimum of 9.3. Preliminary studies suggest identity between trehalose-6-P phosphatase and alkaline phosphatase, the physiological role for which is still not known.

The proposed mechanism of glucose resorption in the kidney may also be applicable to the process of active transport of glucose in the intestinal mucosa. Trehalase and the entire sequence of enzymes leading to synthesis of trehalose are present in the intestinal mucosa.

Proposed Course of Project: Studies will continue to test further the novel hypothesis for active transport of glucose. The role of cations, which are associated with sugar transport will be examined.

Honors and Awards:

Dr. Sacktor was an invited speaker at a seminar of the Physiological Chemistry Department, Johns Hopkins Medical School, Baltimore, Maryland.

Publications: None

Serial No. HD-AG 34
1. Gerontology Research Center
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on the Effects of Temperature and Other Environmental Factors on the Aging Process

Previous Serial Number: HD-AG 34

Principal Investigator: M. A. Brock

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years: None

Project Description:

Objectives: The objectives of this project are two fold: 1) The hypothesis that lowering the body temperature of mammals increases life span will be tested, using golden hamsters, a hibernating species, as experimental animals. 2) While golden hamsters are in deep hibernation, the erythrocyte life span is greatly extended and the concentration of most erythrocyte phosphorylated glycolytic intermediates remains similar to the concentration found in control hamster erythrocytes. Short term (1 hour) in vitro incubation at 4° C of blood from control, cold-exposed, and hibernating hamsters results in substantial changes in the concentrations of phosphorylated intermediates in control and cold-exposed animals.

In contrast, marked changes were not apparent in hibernators' blood. This study will show whether there is an active metabolic adaptation in erythrocytes which takes place at the onset of hibernation, and if this is related to the observed increase in erythrocyte longevity.

Major Findings: No work done in current year.

Proposed Course of Project: 1) Control hamsters will be kept at room temperature (this ensures a body temperature of about 37° C) while another group will be exposed to an ambient temperature of 5 ± 2° C. Most individuals in this cold-exposed group

will hibernate. The life span of hamsters in the cold-exposed, hibernating group will be compared to that of control animals. 2) Glycolytic pathways will be studied using C^{14}-glucose and C^{14}-pentose incubated with blood from control, cold-exposed, and hibernating hamsters at various temperatures. The unidentified ultraviolet absorbing materials in golden hamster erythrocytes will be studied in more detail.

Honors and Awards: None

Publications:

Brock, M. A.: Erythrocyte glycolytic intermediates of control, cold-exposed, and hibernating hamsters. In Fisher, K. C., Dawe, A. R., Lyman, C. P., Schonbaum, E., and South, F. E., Jr. (Eds.): Mammalian Hibernation. III. Edinburgh, Scotland, Oliver and Boyd, Ltd., 1967, pp. 409-420.

Serial No. HD-AG 35
1. Gerontology Research Center
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Changes in Molecular Populations During Development, Maturation, Aging, and Cellular Death

Previous Serial Number: HD-AG35

Principal Investigators: D. D. Hendley (75% time)
B. L. Strehler (40% time) (Left 8/67)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	0.85
Professional:	0.85
Other:	None

Project Description:

Objectives: There is conflicting evidence as to the possible involvement of amino acid activating enzymes in the differentiation of mammalian tissues. Incorporation experiments indicated at least two instances of differences in specific activating enzymes between tissues of the rabbit. On the other hand, a comparison of homologous vs. heterologous deacylation of aminoacyl sRNA indicated no apparent differences among four tissues in activating enzymes for seven amino acids. A more detailed study of the deacylation reaction was undertaken to determine the factors influencing the rate of the reaction, including competing enzymes present in the crude extracts used for tissue comparisons.

Methods Employed: Amino acid activation was assayed by a determination of the incorporation of radioactive amino acid into TCA-insoluble products (forward reaction) or loss of TCA -insoluble counts from labeled aminoacyl sRNA (deacylation reaction).

Major Findings: It was shown that the deacylation reaction is markedly inhibited by both the substrate preparation (aminoacyl sRNA) and product (amino acid). The inhibition at higher RNA concentrations was unaffected by further purification of the aminoacyl sRNA preparation or by increased concentrations of the other components of the reaction. It is concluded that a second RNA binding site may exist on the enzyme which affects its catalytic activity.

Inhibition of deacylation by the amino acid varies markedly among the activating enzymes. The deacylation of alanyl sRNA, for example, is almost unaffected by the presence of alanine, while arginyl sRNA deacylation is inhibited over 50% by 10^{-4} M arginine. Even in the latter case, however, there remains a residual enzyme activity resistant to product inhibition. The mechanism of the inhibition remains unclear. The inhibition appears competitive with AMP, however, the K_I for the amino acid as an inhibitor of the reverse reaction does not correspond with its K_M for the forward reaction.

Competing enzyme activities found in the crude tissue extracts used for these studies include a ribonuclease, cathepsins, pyrophosphatase, and an enzyme splitting pyrophosphate from ATP. This last enzyme increases deacylation in "pyrophosphate-less" control reactions due to the presence of ATP in most of the aminoacyl sRNA preparations.

The resistance of a variable fraction of the sRNA preparations to deacylation even by homologous activating enzyme remains unexplained. It was determined that this was not due to the action of ribonuclease or to the incorporation of amino acid into protein.

It should be noted that the present studies have not brought into question the conclusions reached in the earlier deacylation study, namely, that the specificity of activating enzymes for sRNA species does not vary from tissue to tissue, at least among major sRNA species for seven amino acids in the four tissues studied.

Proposed Course of Project: It is planned to test the hypothesis of translational control of gene repression in a more direct experimental procedure involving *in vitro* protein synthesis. If it can be shown, for example, that polysomes from reticulocytes, rigorously freed from contaminating reticulocyte activating enzymes and tRNA, can synthesize hemoglobin when supplemented only with factors from another tissue, translational control for the synthesis of a tissue-specific protein will have been disproved.

Honors and Awards: None

Publications:

Batra, P., and Strehler, B. L.: Studies on the mechanism of cell death. IV. Alterations in phosphorylative capacity during cell death. Gerontologia, 13: 30-36, 1967.

Strehler, B. L.: Environmental factors in aging and mortality. Environmental Res., 1: 46-88, June 1967.

Strehler, B. L.: The nature of cellular age changes. Symposia Soc. exp. Biol., 21: 149-178, 1967.

Serial No. HD-AG36
1. Gerontology Research Center
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on the Relationship Between Proliferation, Regeneration, Differentiation, and Death of Cells *in vitro* and *in vivo*

Previous Serial Number: HD-AG36

Principal Investigators: B. L. Strehler (Left 8/67)

Other Investigators: R. A. Menzies
R. J. Hays

Cooperating Units: Baltimore City Hospitals; Veterans Administration Hospital, Baltimore, Md.

Man Years: None

Project Description:

This project was discontinued last year.

Honors and Awards: None

Publications:

Hay, R. J., Menzies, R. A., Morgan, H. P., and Strehler, B. L.: The division potential of cells in continuous growth as compared to cells subcultivated after maintenance in stationary phase. *Exp. Geront.*, in press.

Serial No. HD-AG37
1. Gerontology Research Center
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on the Comparative Physiology of Aging

Previous Serial Number: HD-AG37

Principal Investigator: M. A. Brock (66% time)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	0.76
Professional:	.66
Others:	.10

Project Description:

Objectives: Campanularia flexuosa, a colonial Cnidarian, is well-suited for gerontological studies, for its hydranths live for just 10-12 days and then regress. The present study undertakes a description of changes in subcellular structures during the life cycle of the hydranth.

Methods Employed: Electronmicroscopy and electron histochemistry.

Major Findings: Marked modifications of ultrastructure were observed in some but not all cell types of old hydranths. Lysosomes (primary and secondary lysosomes and residual bodies) were seen 1) in the tentacles, in epitheliomuscular cells and neurosecretory cells of the ectoderm and in the gastrodermal cells and 2) in the hydranth body, in epitheliomuscular cells of the ectoderm and in basal areas of digestive cells of the gastroderm. Apically, digestive cells contained more lipid droplets than did these areas in young hydranths. Less marked alterations were seen in the zymogen and mucous cells of the hydranth body gastroderm and in the interstitial cells of the ectoderm. These cell types usually contained a few

single membrane-bounded dense bodies, presumably lysosomes, in addition to subcellular structures unaltered from those of young hydranths. Nematocytes and secretory cells-type 2 were morphologically unchanged in old hydranths.

Preliminary results have shown that nearly all cell types in regressing hydranths contain secondary lysosomes and residual bodies and few other recognizable subcellular structures except for intact nuclei. The nuclei appear unaffected by the massive lytic activity in the cells at this stage. Two cell types, nematocytes and secretory cells - type 2, showed remarkable resistance to the degradative events, for they appeared morphologically unchanged in areas where there was otherwise complete loss of the characteristic hydranth structure.

Proposed Course of Project: The ultrastructural changes in each cell type of regressing hydranths will be described and compared to those of young and old hydranths. Particular attention will be paid to the stages of residual body formation in unusual cell types (nematocytes, for example) and the degree of lytic activity which precedes the formation of regression fragments.

Preliminary electron histochemical studies to describe the localization of acid phosphatase activity will be extended.

Honors and Awards: None

Publications:

Brock, M. A., Strehler, B. L., and Brandes, D.: Ultrastructural studies on the life cycle of a short-lived metazoan, Campanularia flexuosa. I. Structure of the young adult. J. Ultrastr. Res. 21: 281-312, 1967.

Serial No. HD-AG31(8)
1. Gerontology Research Center
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Age-associated Fine Structural Alterations in Cultured Chick Fibroblasts

Previous Serial Number: None

Principal Investigator: M. A. Brock (34% time)

Other Investigator: R. Hay

Cooperating Units: Baltimore City Hospitals; Carnegie Institution of Washington, Embryology Department

Man Years

Total:	0.34
Professional:	0.34
Others:	None

Project Description:

Objectives: The limited growth potential of mammalian and avian diploid cells in culture has been established, and it is suggested that this may be a reflection of senescence at the cellular level. Cells obtained from embryonic chick skin and muscle will undergo a maximum of about 25 divisions. The most obvious morphological modification in late passage cells when compared to early passage cells was the change from spindle-shaped, multilayered cells to rounded, vacuolated dispersed cells in single layers. The purpose of the present study is to investigate further morphological differences that might be present at the ultrastructural level.

Methods Employed: Chick fibroblasts were cultured and passed through either 4.5 or 18 generations. Those cells which had completed 18 generations were considered to be senescent. Cells were fixed by one of a variety of methods and prepared for electron microscopy.

Major Findings: Striking differences along with unexpected similarities between early and late passage cells were apparent. They were consistent regardless of fixation regimen.

		Early passage cells	Late passage cells
1.	Shape of cell	Spindle-shaped.	Most are rounded; a few elongate.
2.	Pinocytosis	Often observed.	Often observed.
3.	Mitochondria	Very large, apparently nearly spherical.	Appear elongate due to a change in shape. The orientation of the cristae changed.
4.	Rough ER	Highly developed.	Highly developed.
5.	Golgi complexes	Numerous.	Numerous.
6.	Single membrane -bounded droplets with inclusions.	Frequently observed.	Either absent or morphologically altered. Appearances suggestive of intermediate stages between the droplets and secondary lysosomes were seen.
7.	Primary and secondary lysosomes.	Few, if any.	Frequent. Polymorphic: may be secondary lysosomes (autophagic vacuoles) or residual bodies.

Proposed Course of Project: Following further evaluation of the electron micrographs on hand, the project will be terminated.

Honors and Awards: None

Publications: None

Serial No. HD-AG29(8)
1. Gerontology Research Center
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Identification of Long-lived Molecular Species

Previous Serial Number: None

Principal Investigators: D. D. Hendley (25% time)
P. Gold (10% time)
B. L. Strehler (60% time) (Left 8/67)

Other Investigators: R. A. Menzies
R. Kutsky

Cooperating Units: Baltimore City Hospitals; Veterans Administration Hospital, Baltimore, Md.; and Dept. of Biochemistry, Louisiana State University, New Orleans, La.

Man Years

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: The early work of Thompson and Ballou indicated that many tissues of the rat contain components in addition to collagen and DNA which have turnover half-lives in excess of 100 days. Since such slowly replaced elements could represent possible loci for senescent changes, an attempt has been made in the present study to identify the unknown components of long half-life.

Methods Employed: Mice were injected at 16 days of age with C^{14}-leucine and subsequently at one month intervals with H^3-leucine. The ratio of the two labels was followed in density gradient subfractions from a variety of tissues for up to 160 days. In a second experiment 2 mice were injected with C^{14}-uridine at 16 days of age and subsequently with H^3-uridine at 105 days. At 141 days of age the RNA isolated from several tissues was pooled and hydrolyzed.

Major Findings: The ratio of C^{14} to H^3 found in a number of tissues of the mice labeled with leucine was indicative of the existence of long-lived protein components, especially in particulate fractions of brain and skeletal muscle, and among the histones of a number of tissues. Insufficient labeled material was available, however, to carry out a planned fractionation to further characterize the proteins involved.

The ratio of C^{14} to H^3 in UMP and CMP isolated from the pooled RNA samples from the uridine-labeled mice could be accounted for only by postulating the existence of a minor RNA component of relatively long half-life (greater than 30 days, compared with 1 to 10 days for known forms of RNA).

Proposed Course of Project: Using larger numbers of mice to obtain sufficient material, an attempt will be made to determine the nature of the long-lived RNA species and its tissue localization.

Honors and Awards: None

Publications: None

Serial No. HD-AG32(8)
1. Gerontology Research Center
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Lipofuscin pigment accumulation as a function of age and localization in animal tissues, and its pathophysiological significance.

Previous Serial Number: None

Principal Investigator: W. Reichel (100% time)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	2.5
Professional:	1.0
Others:	1.5

Project Description:

Objectives: (1) The quantitation of lipofuscin pigment with relation to age and specific localization within organs; (2) The correlation of pigment content with physiological capacity of the cells.

Methods Employed: A grid-counting technique and fluorescence -phase microscopy were used to measure pigment accumulation in various tissues. A histochemical method was developed for 3 beta-hydroxy steroid dehydrogenase, an enzyme necessary in steroidogenesis. Cell diameter, nuclear diameter, cortex width and zona reticularis width were studied in the rat adrenal cortex using filar micrometry.

Major Findings: 1. Lipofuscin pigment accumulates at markedly different rates in specific areas of rat and mouse brain as a function of age. The hippocampus of the senescent Wistar rat and C57BL/6J mouse selectively accumulates more pigment per section volume (5%) than the cerebral cortex and the Purkinje layer of the cerebellum. By contrast, in the granular cell

layer of the cerebellum in the old rat and mouse, less than 0.6% of section volume is occupied by pigment. Although the hippocampus is the most heavily pigmented area in terms of percent of section volume, the Purkinje layer of cerebellum of the senescent Wistar rat has the highest proportion of intracellular volume occupied by pigment (17.5%).

2. In the senescent male and female Wistar rat, the parenchymal cells of the zona reticularis of the adrenal cortex, the ovarian macrophages and the testicular interstitial cells of Leydig accumulate much more pigment per cell than other cell types or zones studied in five organs (19.2-22.6%, 53.5, 15.1%, respectively). All other cell types studied contain less than 4.5% pigment per intracellular volume, and a total absence of pigment was noted in the maturing ovarian follicle, in the seminiferous tubule of the testis, and in the renal glomerulus.

These results suggest that lipofuscin accumulation occurs to a greater degree in slowly-dividing or post-mitotic cells, such as neurons, and to a lesser degree in replicating cells or cells with a short half-life, such as the developing cells of the ovarian follicle or within the seminiferous tubule. These results also suggest that the formation of pigment is related to the metabolism of the ketosteroid precursors or ketosteroids stored in the adrenal cortex, the ovary and the testis.

3. Preliminary qualitative study of 3 beta-hydroxy steroid dehydrogenase activity in the inner zone of adrenal cortex suggests that cells are stained more intensely in the younger animals than in the senescent group (which contain heavy lipofuscin accumulations in this zone).

4. Abundant lipofuscin pigment was noted in the major organs of two children with progeria who recently died. The pigment appeared in the same cell types in which it is found in the senescent rat. This is the first demonstration of lipofuscin accumulation in this clinical disorder.

5. In a comparison of mature and senescent rats, both cell diameters (9.8 ± 0.21 and 9.7 ± 0.22 μ, respectively) and nuclear diameters (5.3 ± 0.06 and 5.3 ± 0.04 μ) are not significantly different in the zona reticularis. Nor are cortex width and zona reticularis width significantly different in young and old animals.

6. A lipofuscin-like pigment is found in the same location in the adrenal cortex of 3 month old vitamin E-deficient rats as in normal senescent rats. Counts in the zona reticularis are: 2.9 ± 0.28%, E-deficient 3 month old rat; 0.4 ± 0.14%,

E-sufficient 3 month old rat; 4.3 ± 0.42%, 12 month old rat on a standard diet. Although lipofuscin-like pigment has been described previously in vitamin E-deficiency, this study quantitates pigment as a function of vitamin E diet, age, and specific localization of pigment in the adrenal gland.

Proposed Course of Project:

1. A television scanning system has been developed for quantitating the results of histochemical reactions. It will be used to evaluate assays for 3 beta- and 17 beta-hydroxy steroid dehydrogenase activities. An attempt will also be made to estimate lipofuscin pigment volume in a series of human tissue sections.

2. A larger series of progeric patients is planned, with a control series of normal human autopsy specimens for quantitation of lipofuscin in major organs. The planned investigation will attempt to document whether or not these children exhibit four characteristics of human aging: lipofuscin accumulation; collagen changes; arteriosclerosis; and physiological decrement.

3. A larger number of vitamin E-deficient rats will be studied as to localization and quantity of lipofuscin pigment, and results will be correlated with vitamin E tissue levels (in cooperation with Dr. W. Weglicki).

Honors and Awards: None

Publications:

Reichel, W.: Why do people grow old? Resident Physician, 14: 86-92, Jan. 1968.

Reichel, W., Hollander, J., Clark, J. H., and Strehler, B. L.: Lipofuscin pigment accumulation as a function of age and distribution in rodent brain. J. Geront., 23: (1), 71-78, Jan. 1968.

Reichel, W.: Lipofuscin pigment accumulation and distribution in five rat organs as a function of age. J. Geront., 23: (2), 145-153, April 1968.

Serial No. HD-AG33(8)
1. Gerontology Research Center
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Turnover of Subcellular Components

Previous Serial Number: None

Principal Investigator: P. Gold (90% time)

Other Investigator: R. A. Menzies

Cooperating Units: Baltimore City Hospitals; Veterans Administration Hospital, Baltimore, Md., and Dept. of Biochemistry, Louisiana State University, New Orleans, La.

Man Years

Total:	3.05
Professional:	0.9
Others:	2.15

Project Description:

Objectives: The general objective of the work is the elucidation of the dynamic changes occurring in cellular constituents in the course of development and aging. Within this general framework, a study has been initiated of the synthesis and turnover of ribosomal and other RNA species and mitochondria in the rat.

The studies of Wulff et al. suggested that some species of RNA are synthesized at higher rates in old animals than in young. It was proposed that increased RNA and protein turnover might be the response of a feed-back mechanism to the possible production of defective enzymes in the senescent organism. The studies of Devi et al., on the other hand, suggest that nuclear RNA synthesis is slower in the old rat than in the young. Both these studies suffer from the fact that the results are strongly influenced by differences in pool sizes of intermediates and in distribution of the isotope throughout the organism.

We therefore turned to a study of the degradation rate of

in vivo-labeled RNA as a more valid measure of RNA turnover free from the above objections. To avoid confusion each type of RNA would be separately isolated and measured. By simultaneously labeling with a second isotope (H^3-leucine) it was possible to measure the degradation rate of ribosomal protein and mitochondria in the same experiment.

Methods Employed: Six, twelve, and twenty-four month old rats were injected with H^3-leucine and C^{14}-orotic acid in order to label both protein and nucleic acid components. The specific activities of the ribosomes and mitochondria isolated from a number of tissues was determined at intervals up to 37 days after labeling.

Major Findings: A) The decay curves of mitochondrial turnover in different tissues could be divided into two classes: (1) single exponential decay: (a) liver, t1/2 = 8.4 days, (b) brain, t 1/2 = 24 days, (c) testes, t 1/2 = 10.0 days; and (2) double exponential decay; (a) lung, t 1/2 = 4.6 and 14 days, (b) kidney, t 1/2 = 4.3 and 11.6 days, and (c) intestinal mucosa, t 1/2 = 0.9 and 10.5 days. No significant differences were found between the different age groups. This confirms the results of Fletcher and Sanadi on liver mitochondria and extends the work to other tissues. The early component of the double exponential decay group may reflect the turnover of different populations of cells in the tissue, or possibly the independent turnover of certain protein components of mitochondria. B) The turnover data for ribosomal RNA have not yet been treated statistically and are therefore still preliminary. Apparently single exponential decay curves were found for liver (t 1/2 = 6.0 days), kidney (t 1/2 = 6.5 days), lung (t 1/2 = 9 days), and spleen (t 1/2 = 9 days). No obvious differences could be observed between the different age groups.

In the case of brain, the specific activity of ribosomal RNA did not reach a maximum until approximately the sixth day after labeling. The decay curve appears to be complex. There appears to be an age difference in the maximum specific activity reached. The brain ribosomal protein decay curves appear to parallel the RNA curves. The special features of brain metabolism, such as the blood-brain barrier, make interpretation difficult, however.

Proposed Course of Project: Work proposed for the coming year includes: (1) completion of ribosomal RNA turnover studies, (2) a study of tRNA and nuclear RNA turnover, (3) determination of the turnover of the total ribosomal particle and its subunits, (4) further study of RNA turnover in the brain using direct injection of label into the ventricular space, and (5) a study of the turnover of lipofuscin in the human liver and myocardium utilizing autopsy material and the marked increase in

atmospheric C^{14} since 1960.

Honors and Awards: None

Publications: None

Serial No. HD-AG38
1. Gerontology Research Center
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Fractionation of Aging Connective Tissue

Previous Serial Number: HD-AG38

Principal Investigator: H. R. Elden

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years: None

Project Description:

Objectives: Collagen undergoes a series of chemical reactions (crosslinking) which transforms soluble molecules into insoluble fibers. There are numerous ways that intermolecular crosslinkages might form; however, if solubility depends only upon molecular weight then the specific type of crosslinking reaction should not influence solubility. The serial extraction of components from a collagen matrix, therefore, could be regulated mainly by two parameters: viz., (a) the number of molecular fragments having molecular weight, MW_i, and (2) the change in molecular weight with serial depletion, $MW_i(n_i)$, where n_i is the number of serial extraction.

The objective of this study is to ascertain how collagen is serially depleted from a fibrous matrix under conditions that favor solubilization. In particular, one hopes to find a sharp demarcation between labile and refractory collagen fragments; should it not exist then the finding would be equally valuable. A second objective of this study is to determine the number of components obtained by a standard extraction procedure, as well as the molecular weight of each such component. This information could then be used to construct a serial elution concentration-profile for collagen fibers. A nomogram of this type then would be a rapid way to chronologically follow polymerization of collagen fibers during growth-development, aging, and pharmacologic control.

Methods Employed: Samples of fetal calf skin were extracted with hot ethanol to remove fat, then soaked in acetone, evacuated to constant weight, then stored in a dessicator. One hundred milligram samples were serially extracted with 3 mls. of water for 30 minutes at temperatures of 40, 50, 60, 65, and 70°C. The optical density of 250 millimicrons and that of the tyrosine reaction product (Folin-Ciocalteau) were used to follow the serial extraction. Residual protein was then autoclaved serially. Finally, a series of autoclaved digests were made on samples which had not been serially extracted.

Proposed Course of Project: No progress was made on this project due to limitations in personnel and lack of equipment needed to measure molecular weights of solubilized components. Recently acquired instrumentation will permit the rapid determination of molecular weights, i.e., direct reading osmotic pressure manometers. An attempt will be made to reduce the exceedingly large quantity of samples needed to be tested to a series of technical operations capable of being executed by semi-skilled assistants. The need to obtain statistical data with regard to age, sex, animal number, and sample reproducibility all greatly accentuate the work involved in what otherwise would be an 'apparently' simple measurement to make.

Honors and Awards: None

Publications: None

Serial No. HD-AG39

1. Gerontology Research Center
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Elasticity of Aging Connective Tissue

Previous Serial Number: HD AG-39

Principal Investigator: Harry R. Elden (50% time)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives: Previous reports on this project have shown that longitudinal elongation and stress of rat tail tendon are related by an equation which yields (K_C) as a modulus of elasticity. This term then was shown to decrease logarithmically with increasing sample cross sectional area (W_O). Constants obtained from plots of log K_C vs. log W_O changed with aging and suggest that elastic stiffness of thin tendons decreased whereas stiffness of thick tendons increased with aging. Additional findings of previous reports indicate that cohesion and cross sectional mass (W_O) increased with growth and aging; velocity of tendon depolymerization to break (T_B) increased linearly during growth but then increased exponentially with aging when early growth period was attenuated.

The present report extends these observations by precisely measuring the age dependence at close intervals. Additional measurements on tensile properties, cohesion, and melting were obtained to evaluate sensitivity of these tests by statistical procedures.

Methods Employed: Cohesion of tail tendon was estimated by measuring the cross sectional mass W_O (mgs./cm.) as described

previously. In the present study, 20 tendons from each tail of 6 rats (male and female) were studied at 4, 6, 8, 10, and 12 weeks, followed by another run at 1, 3, 6, 12, 18, and 24 months of age. Body weight was measured on each animal so that parameters of findings in A, B, C, and D (below) could be interpreted with regard to growth and aging processes.

Elasticity of skin was determined by a new technique, but it utilized rats appropriated for assessment of dehydration reported in HD AG-39. A ring of tissue was dissected by a circumcision of the center of each rat tail. This sample was stored in the freezer without chemical treatment. An optical measuring device was used to determine sample length and thickness. Force and longitudinal extension were evaluated with the Instron tensile testing apparatus; samples were placed over two right angle hooks attached to the strain gauge and stretching assembly; tissues were hydrated by admitting water from a constant temperature water bath maintained at 40°C.

Time-to-break was measured in 5 M urea at 40°C as previously described. Multiple testing devices were used in this run to expedite collection of data.

Major Findings: A. Cohesion of rat tail tendon. The maximum length of isolated tendon (L_{max}), that tendon which approximated the entire length of the tail, showed rapid growth during the first 3 months; a steady value was approached between 3 to 24 months of age. L_{max} was not linearly dependent upon body weight, but it increased rapidly during growth to 100 grams then slowed down as the body weight reached 500 grams. It never approached a steady value with respect to body weight as closely as it did with respect to age. Weight of each tendon (W) decreased approximately linearly with respect to increasing tendon length (L). Long tendons weighed less than short ones. The cross sectional mass $W_o = (W/L)$ likewise decreased with increasing L. Finally, a linear relationship with positive slope was established between $-dL/dW_o$ vs. L_o^2. These graphs state that the rate of decrease in tendon cross sectional area with respect to a function of increasing length, i.e., $(-dL/dW_o)$ is a function of L_o, viz., $(K4 + K_5L^2)^{-1}$. As L_o gets bigger the decrement $-dW_o/dL$ gets smaller, L_o is a theoretical abstraction of the data for W_o vs. L, but L_o is related to real parameters; viz., $L_o = K_2/K_3$, where $K_2 = W^o/L$ and $K_3 = W_o/L$. L_o shows a rapid increase during the first month of age, but thereafter it fluctuates extensively about a central value. Thus, beyond 1 month there is fluctuation in $-dW_o/dL$ with respect to age. Fundamental equations are relevant to the above findings:

$W = W^{O} - K_1 L$

$W_o = K_2 - K3\ L$; the slope $K_3 = dW_o/dL$; and $W_o = \frac{W}{L}$

$-dL/dW_o = K_4 + K_5 L_o^2$; L_o is defined as that l at which $W_o = O$ by extrapolation.

Mean values of W_o increased rapidly during 1 to 3 months of age, but values thereafter fluctuated about a tendency for further increase during 3 to 24 months of age. Mean W_o also increased linearly with changes in body weight for all age data. A linear relationship was discovered between standard deviation of W_o and mean W_o for the 20 tendons examined in each tail.

A theoretical basis for this interrelationship was not developed but it was established that the spread in standard deviation W_o with respect to mean W_o reflects a change in distribution of sizes of the 20 tendons. A frequency vs. size distribution was computed based on 0.10 increments of W_o for 20 tendons from each tail. Mean and standard deviation of the number of tendons per interval were computed for animals comprising each age and sex group. The number of tendons of the small size interval increased for a peak, then decreased with respect to age (4 to 12 weeks); the middle size groups became filled after the increase-decline of the smallest group. A steady state level of tendon numbers was observed in the middle size group that lasted for weeks. Finally, this steady state declined after which the largest size groups became filled with numbers of tendon (3 to 24 months).

The distribution function was skewed toward large sample size; increase in standard deviation W_o vs. W_o^2 depends on the increase in skewness. Most of the age changes occurred between 4 to 12 weeks, with fluctuation being accounted for on the basis of body weight. Old tendons contrast with young tendons by having wider distribution of tendon size and higher mean value of W_o.

B. Stiffness of rat tail tendon. The modulus of elasticity K_C decreased logarithmically with increasing cross sectional mass W_o (mgs./cm.) of tendon, viz., $\log K_C = A - B \log W$, for 5 samples of 3 male and 3 female rats at 2, 6, 12, 18, and 24 months of age. Slope B and intercept A drifted downward during this age period. The most outstanding change with age occurred in yield and rupture stress. Both of these tensile properties increased steadily between 4 weeks and 24 months without appreciable sex difference. Subsequent evaluation of data revealed, however, that yield stress (F_Y/W_o) and rupture stress (F_B/W) increased linearly with cross sectional mass W_o, even though

force data had been corrected for mean cross sectional mass. It appears that reduction in stiffness (K_C) with mean cross sectional mass (W_o) is accompanied by an elevation of tensile strength. Tensile strain to yield and rupture were independent of W_o and invariant with respect to age. Influence of aging on these properties is due principally to an increase in W_o.

C. Time-to-Break (T_B). Depolymerization of tendon to rupture in 5 M urea (40° C.) was determined on 20 tendons from each tail of two runs; (1) 6 male and 6 female rats at 3, 6, 12, 18, and 24 months, and (2) 3 male and 3 female rats at 3, 6, 12, 18 and 24 months. These repeated measurements confirmed findings previously obtained. Mean T_B increased linearly with respect to body weight throughout the 0 to 200 gram interval. Beyond the 200 gram weight interval, T_B increased exponentially in excess of the linearly extrapolated values. Departure from initial linearity occurred at 200 grams in females and at 300 grams in the males. These weights correspond to ages wherein rapid initial growth begins to slow down, viz., 3 months. Evaluation of T_B vs. age shows a continuous increase, but the functional dependence of T_B vs. age is less obvious than is T_B vs. body weight. The two runs made on this test, even though confirming previous findings, were necessary to firmly establish reproducibility of the linear and non-linear dependence of T_B vs. body weight.

Of all the test parameters so far studied, T_B is the most sensitive, reproducible, and feasible for large scale assessment of aging effects in connective tissue of an animal colony. The great sensitivity of T_B vs. age is that it increases exponentially with respect to body weight during the age interval (3 to 24 months) where other properties become stable.

D. Tensile properties of aging dermis. Growth curves showed that 24 month male rats weighed considerably less than expected; this is a confirmation of previous observations that weight of males begins to decline at 18 months while females retain constant weight until past 24 months. Several other animals showed erratic positions of data which necessitated some selection of data when statistical evaluations were conducted.

Skin thickness increased progressively with age and reached a constant level at 6 months; fluctuations thereafter and the preceding growth period all were related linearly to body weight. Deformation induced by cycling load to 100 then to 500 grams showed an increase with age; correlation with body weight was erratic. Yield and rupture stresses increased with age most rapidly between 1 and 3 months with a further increase between 3 and 24 months, but at a lower rate. A linear trend existed between these tensile parameters and body weight including all

ages. Yield and rupture stresses also were correlated linearly with each other. Finally, the modulus of elasticity (stiffness), K_c, was computed after demonstrating that data obeyed the equation $F = K_C (l - 1)^2$. K_C increased rapidly between 3 to 6 months, then continued to increase slowly thereafter (6 to 24 months), but it was low and essentially constant during early growth between 1 to 3 months.

Proposed Course of the Project. A-B. The mechanism of longitudinal elastic stiffness and lateral cohesion will be investigated further using pulse propagation techniques. Orientation as well as elastic modulus and density of fibers influence the transit time of a standardized sonic pulse. Samples will be stretched longitudinally during which the pulse propagation time will be simultaneously ascertained. The experimental data then will be used to correlate changes in tensile stiffness with changes in orientation of fibers in the stretching tendons. C. Time-to-break will be used in studies designed to ascertain influences of specific compounds and nutritional status on cohesion of collagen at the molecular level. We are particularly interested in compounds related to pencillamine and food restriction as factors which might retard or reverse cohesion of aging tendon fibers. D. Tensile properties of aging dermis will be extended by investigating the change in parameters as samples are dissected at different positions along the length of the rat tail. Thermal degradation of these rings of tissue will be used to determine the crosslink density of the collagen chains made amorphous by heating above the melting temperature of the collagen crystallites. These data will be used to determine whether or not there is a progressive change in properties of collagen from tip of the tail to base of the tail, i.e., distal to proximal aspects, as a recollection of aging effects imposed on the animal since birth.

Honors and Awards: None

Publications: None

Serial No. HD-AG40
1. Gerontology Research Center
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Hydration of Aging Connective Tissue

Previous Serial Number: HD AG-40

Principal Investigator: Harry R. Elden (20% time)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years:

Total:	.70
Professional:	0.20
Others:	.50

Project Description

Objectives: Previous reports in this series on mechanisms of water binding in connective tissue used samples of tissue that were dired and extracted with solvents for fat. Subsequent rehydration then yielded data on equilibrium multilayer adsorption and desorption. The parameters used to evaluate these measurements did not show very great response to age, although the measurements contributed to understanding how water is bound to the fibrous matrix of skin. This project was extended by measuring the rate and velocity of dehydration of freshly dissected rat skin, without any previous chemical treatment. This technique does show an age-related change in the interaction of water with fibrous components of skin.

Methods Employed: An instrumental set-up was constructed that permitted continuous weighing of samples maintained in a stream of 0 relative humidity air. Circulating water from a water bath maintained the sample chamber and air stream at 40° C. Wistar rats were selected from the colony of aging rats of the Gerontology Research Center to make up the following series of male and female groups: 3 animals of both sexes at 1, 3, 6, 12, 18, and 24 months. After sacrificing by exposure to ether, each animal was weighed and clipped of hair along the centerline of

of his back. A rectangle of skin approximately 1 cm. wide by 2 cm. long was dissected out, rapidly weighed, and suspended by wire into the atmosphere of dry air. Weighings were made serially until samples attained constant weight.

Major Findings: Rate of dehydration. Non-linear reduction in sample weight required 48 hours to approximate equilibrium with 0 relative humidity air. The following description parameters were used to succinctly express the empirical data:

W_t = transient weight; W_{oo} = final weight, W_o = zero-time weight; $Q = W_o - W_{oo}$; $q = W_t - W_{oo}$ Q/W_{oo} = total water weight/dry sample weight; and first half life. Computations of 1 - F, 1/F and F/T, and F, were made to establish functional inter-dependence of the experimental data. $F = (W_t - W_{oo})/(W_o - W_{oo})$.

Plots of F vs. time showed concave departure from linearity. Deviation was least for 1 month age samples, and it increased abruptly at 3 months. Rate of dehydration therefore was slowest in the 1 month age group but increased significantly at 3 months. Curves of F vs. time for 3 to 24 months fluctuated about a central tendency. However, individual aspects of the velocity of dehydration (3 to 24 months) were related more to Q and half-life. Those samples which had the longest half-life, the young tissues which deviated least from the linearity, also had the highest content of extracted water. Thus, a plot of half-life vs. Q/W_{oo} increased very rapidly from 50 to 350 minutes as the ratio of water weight to sample dry weight increased from 1.20 to 3.00.

Age and body weight. Water content of tissues decreased from a mean 3.5 to 1.5 between 1 and 3 months; subsequent aging (6 to 24 months) only decreased this figure to values between 1.40 to 1.50. Half-life for rate of dehydration decreased markedly during the 1 to 3 months age period, whereas it fluctuated about a mean value during the 3 to 24 months age period. Replotting data vs. body weight shows a greater dependence than exists with respect to age.

Mechanism of water binding and dehydration. Water is retained in the fibers of skin by condensation in capillaries and by multilayer adsorption on reactive surface sites. The potential for water to boil-off from the condensed state in skin to the passing 0 relative humidity air stream depends upon its location relative to open surface in capillaries or reactive (polar) sites. Those molecules that are located farthest from an adsorption site have the greatest potential for escape. It is reasonable to expect, therefore, that rate of dehydration will decrease in some manner related to the quantity of water left on the surface, or in the skin. Appropriate computation of data

then yielded a plot of F/t, (Q) F/T and F Q at intervals of F separated by one-tenth. It was established that data from male and female tissues 3 to 24 months of age conformed to a smooth curve relating velocity of dehydration to water content. Instantaneous velocity of dehydration, therefore, depended strongly upon water content. This graph occluded the fluctuations originally observed with respect to body weight and older age. The linear desorption of water from 1 month tissues showed a much lower velocity, greater half-life than accounted for by this analysis. The velocity of desorption nevertheless varied linearly with water content at its lower slope.

Proposed Course of Project: The kinetic studies reported here will be supplemented by desorbing water to equilibrium with atmospheres of fixed relative humidity. Equilibrium weight of samples with serial reduction of equilibrium relative humidity will be measured and used to construct an equilibrium-desorption isotherm. Analysis of the desorption isotherm by multilayer adsorption theory will attempt to determine the number of layers of water molecules adsorbed to fibrous surfaces, and the size of the surface in meters^2/gram of dry tissue will be estimated. These parameters will be related to the chemical composition of tissue during its growth and aging.

Honors and Awards: None

Publications: None

Serial No. HD-AG43
1. Gerontology Research Center
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Endocrine regulation of connective tissue

Previous Serial Number: HD-AG43

Principal Investigator: H. R. Elden (30% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years
Total: .80
Professional: .30
Other: .50

Project Description:

Objectives: This study concludes the project started (HD-AG43, 1967) in search of a test of biodynamicity of connective tissue as a function of age. The original design of imposing an endocrine disturbance on rats at various ages followed by serial analysis was continued in the past year's work. This report, therefore, deals with response of female rats to hypophysectomy, imposed at various ages, and followed serially with respect to body weight and tendon cohesion.

Methods: The influence of hypophysectomy on tendon cohesion was ascertained by measuring W_O and T_B; these were related to body weight of control and experimental animals. Technical details for these measurements followed exactly those described in previous and concurrent project reports.

Major Findings: Mean values of W_O increased linearly with body weight and all data fell on a line which included 6 control and 6 experimental groups. Range of weight extended from 75 to 275 grams over an age span of 9 months. Hypophysectomy significantly reduced W_O in the two youngest (1 and 2 months) and the two oldest (6 and 9 months) age groups in proportion to difference between control and experimental body weight. Time-to-break (T_B) was higher in the two youngest groups of

hypophysectomized rats compared with their controls; no significant differences were found in the older groups. Generally, there was a tendency for mean values to be displaced toward lower body weight; viz., the experimental group was displaced toward the left on the weight axis.

Other comparisons of data, i.e., standard deviation of W_o vs. W^2 and standard deviation of T_B vs. T_B, revealed that findings were consistently following empirical relationships observed in other animal tissues (Wistar rats at 1 to 24 months) whereas endocrine disturbances were not imposed; the only difference attributable to endocrinologic factors were the coordinates of test parameters on the empirical graphs. Removal of the pituitary reduces velocity of body growth, although it does not obliterate it entirely in young rats. Short term serial reaction (2 months) to hypophysectomy induced at various ages between 1 and 9 months changes the cohesion of collagen fibers in tendon in proportion to changes produced in body weight. Reduction of W_o is interpreted as depletion of protein in general; the fractional loss in W_o of hypophysectomized rats, compared to their controls in young rats, probably is determined by the size of labile collagen pool in the fibers. The molecular cohesion (T_B) of the residual fiber increases because there is an increase in the fraction of tendon that is metabolically inert, e.g., refractile and presumably highly crosslinked. Short term reaction to hypophysectomy at 9 months of age does show a lower mean T_B, but it is not significant statistically; all other values of T_B are high in experimental compared with control groups.

Frequency vs. size histograms of tendon W_o show that hypophysectomy reduces the number of large tendons; concurrent reduction of coordinates on plots of standard deviation W_o vs. W_o^2, therefore agree with interpretations of this graph for tendon changes produced by growth and aging.

Proposed Course of Project: This study completes the planned course of investigation. Many goals were not attained, however, due to technical limitations of personnel. Future work along this line will not be attemped until animal facilities become available. At this time we will investigate the influence of supplementary growth hormone on cohesion of tendon fibrils. Specifically, we want to know if there is an age beyond which cohesion of fibers and body weight do not change even when supplementary growth hormone is present.

Honors and Awards: None

Publications: None

Serial No. HD-AG37(8)
1. Gerontology Research Center
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Enzymes of the Elastase Complex in the Vessel Wall

Previous Serial Number: None

Principal Investigator: W. A. Loeven (E.O.D. 10/11/67)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	0.66
Professional:	0.66
Others:	None

Project Description:

Objectives: In the last few years more details have been given by several authors about the possible intervention of the enzymes of the elastase complex (elastoproteinase and elastomucase) in the onset and/or control of arterial lesions and the influence of age on the enzyme production in the pancreas. However, all the theories put forward depend on the assumption that the pancreatic enzymes pass into the vascular tissues via the circulation in this way performing a systemic function. Several observations indeed suggest that the enzymes may be secreted into the pancreatic juice, become readsorbed into the intestinal mucosa and circulate in the blood. Their presence was already demonstrated in blood. Investigations concerning their presence in the vessel wall itself were started when the present investigator was still employed by the Netherlands Institute for Preventive Medicine, Leiden, The Netherlands.

Methods Employed: Cow and calf aortae obtained from the slaughterhouse were cut into small pieces after removing of the adventitial material, washed with tap water to remove the blood, minced, extracted with acetone, dried and finally minced in a kind of hammer mill. Part of the dried powder was directly brought onto columns and column chromatography techniques were

performed to elute several protein containing fractions. Part of the powder was first extracted with 0.9% NaCl, 5% EDTA, Teorell-Stenhagen buffer of pH 10.7, and acetate buffer of pH 4.7, dialyzed and freeze-dried. These four extracts were treated in the same way as the whole aorta powder during the column chromatography procedure. All the eluted fractions were dialyzed and freeze-dried. In the dried fractions the eventually present enzymes elastoproteinase and elastomucase and the elastoproteinase inhibitor were measured with our standard methods. Under the experimental conditions of the enzymic methods used, an elastoproteinase activity of 0.02 units/mg dried protein and an elastomucase and elastoproteinase inhibitor activity of 0.05 elastomucase units/mg and 0.04 inhibitor units/mg, respectively, lie just out of the limits of the experimental errors.

Major Findings: The results of the experiments performed with calf and cow aorta powder clearly demonstrate that small but consistently present amounts of the enzymes elastoproteinase and elastomucase and of the elastoproteinase inhibitor can be identified, both with the routine methods for determination of enzyme and inhibitor activities and with the standard paper electrophoresis techniques. The third enzyme of the elastase complex, the lipolytic component elastolipoproteinase (releasing primarily the lipid material in the elastic fibres) seems to be also present, but this could not be definitely proved.

The results of this investigation which must be considered as a preliminary experiment suggests that an age relationship in the content of enzymes and inhibitor cannot be excluded. In calf aorta the yield of elastoproteinase appears to be somewhat higher than in cow aorta (from 3 year old animals); yield expressed in mg protein per mg original dried aortia powder. The same trend is also present for the elastomucase and the elastoproteinase inhibitor contents, although less pronounced than for the elastoproteinase. If the elastolipoproteinase found in the crude extract with 5% EDTA is the same enzyme fraction as previously isolated from pancreas and blood and not contaminated with the elastomucase, its concentration in cow aorta is much higher than in calf aorta. If this result can be repeated in experiments to be started later, this finding may be very important for the theory that the enzymes of the elastase complex are indeed involved in the processes of aging and atherosclerosis. In the older vessel wall there is much more metachromatically stainable carbohydrate material and lipid deposits than present in the young tissue, while at the same time there are drastic changes in the structure of the connective tissue of the media and intima parts of the vessel wall.

Proposed Course of Project: Although small amounts of elastoproteinase, elastomucase, and elastoproteinase inhibitor could be measured in bovine aorta, the enzymic activities of the isolated fractions were less than expected from the work done with the pancreatic enzymes. Two suggestions have been given for these findings: a. the enzymes are still bound to a lipopolysaccharide carrier in the vessel wall; b. the tissue enzymes are more labile than the pancreatic ones and become partially denatured during the extraction and chromatographic procedures. This might perhaps also explain the phenomenon that the fraction which shows elastolytic activity, and at the same time also inhibits the activity of highly purified pancreatic elastoproteinase.

It will be tried to refine the techniques used in order to avoid these contaminating effects or in any case to get more information about these findings.

Honors and Awards: None

Publications: None

Serial No. HD-AG45(8)
1. Gerontology Research Center
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Influence of Age on the Collagenolysis and Elastolysis of Lung, Heart, and Aorta by the Enzymes of the Elastase Complex Using Male and Female Rat Tissues

Previous Serial Number: None

Principal Investigator: W. A. Loeven, Ph.D. (E.O.D. 10/11/67)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years: None

Project Description:

Objectives: On the basis of the fact that elastogenesis is usually associated with the presence of large amounts of metachromatic and PAS-positive material, several authors have argued that the physical properties of elastic tissue may result from the macromolecular structure of elastin and the way in which these elastic fibers are cemented by collagenous structures, mucoproteins and mucopolysaccharides, lipoprotein-like material, fluorescent compounds and other pigments. The heterogeneity of the carbohydrate moieties present in elastin has already been mentioned.

Pigments and fluorescent compounds are being intensively studied by various groups of workers, who believe that these substances might represent a kind of gross-linking: a mechanism of in vivo "tanning" which seems to be age-correlated.

It must, however, always keep in mind that the structure of elastic tissue can only be maintained by a complex balance of the elastolytic enzymes and their inhibitors. Any slight change in this balance may disturb these structures. Here it is not the place to give a detailed survey of the data found in the literature concerning the aging of elastic tissue and elastin and the changes in the elastic structure in some diseases affecting it.

The following subjects have been studied: the correlation between aging and the dissolution of elastic fibres by "elastase"; the effect of elasto-mucase on the elastic extensibility of arteries in relation to age and

atherosclerosis; the relationship between "elastase" activity in the pancreas and the content of pulmonary elastin and degree of chronic pulmonary emphysema; and the susceptibility of elastic fibres in pseudoxanthema elasticum and in senile elastosis to "elastase".

However, most of the experiments mentioned above were done with partially purified enzyme preparation (the reason that we have written "elastase" is because no data are available about the purity of the enzyme). At the present time we know that at least four enzymes belong to the "elastase complex": elastoproteinase, the proteolytic elastase component which solubilizes the elastin protein; the elastomucases Em-I and Em-S which primarily attack the carbohydrate moieties in the elastic tissue and isolated elastin (the reaction mechanism of these two elastomucases is not the same); and finally the elastolipoproteinase, the lipolytic elastase component which releases lipid like material from the elastic fibres. All these elastolytic enzymes can be isolated and purified routinely and are available for the project program to be described in the next section.

The objective of the study will be to determine in how far the connective tissue components of lung, heart, and aorta change with age, not only with regard to their own composition but also to their susceptibility to the enzymes mentioned above and collagenase.

Further it will be determined to what extent the composition of the collagenolysates and elastolysates obtained during the incubation of the tissues with collagenase and the enzymes of the elastase complex changes with age.

Methods Employed: Lung, heart, and aorta tissues from male and female rats will be used. 240 male and 240 female animals are necessary, namely 25 males and females in the age groups of 1, 2, 3, 4, 5, and 6 months old and 15 animals of each sex in the age groups of 8, 10, 12, 16, 20, and 24 months old.

The amounts of lung tissue (and perhaps also of the heart) which can be collected is enough to perform the experiments using the methods described in the next section. The amount of aorta tissue is far too small to do all this work and here we must make a decision what determination will be performed and what will be omitted.

Lung, heart, and aorta of male and female rats will be removed and treated in the following way:

a. in the whole tissue we will determine:
   1. the content of non-scleroproteins;
   2. the content of soluble collagen;
   3. the content of insoluble collagen;
   4. the content of elastin

In the fractions obtained in this way a further characterization will be made by measuring the contents of:

1. hydroxyproline;
2. hexosamine;
3. hexose;
4. hexuronic acid;
5. NANA (Sialic acid).

b. After removing of salts and the non-scleroproteins, the reamining connective tissue in lung, heart, and aorta will be incubated with collagenase and/or elastoproteinase, elastomucase, and elastolipoproteinase in order to measure the susceptibility of the tissues to these enzymes.

If the collagenolysates and elastolysates obtained from these tissues at (if possible) two enzyme concentrations the following determinations will be performed under the restrictions to be made in the next section:

1. total protein content;
2. hydroxyproline content;
3. hexosamine content;
4. hexose content;
5. hexuronic acid content;
6. NANA content

c. If it is possible it is our intention to try to measure the release of fluorescent material from the tissue during the incubation with the various enzymes with the methods which was already partially developed in Leiden, Holland (Netherlands Institute for Preventive Medicine).

Restriction to the experiments described above:

A. The determinations of hexosamine, hexose, hexuronic acid, and NANA concentrations in the collagenolysates and elastolysates will only be performed if the amounts present are high enough to use the routine methods previously used.

B. Elastolipoproteinase will only be used in these experiments if we succeed in preparing a new active enzyme sample. From the other enzymes the amounts present are large enough to do this work.

Major Findings: None. This project will be started this summer. Up till now only the methods to be employed in this project (mentioned under a.) have been tested for their usefullness.

Proposed Course of the Project: Since this is a new project to be started in FY 69, the course of the project is as described above under project description.

Honors and Awards: None

Publications: None

Serial No. HD-AG44(8)
1. Gerontology Research Center
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Influence of Age on the Content of the Enzymes of the Elastase Complex in the Pancreas and the Blood of Rats.

Previous Serial Number: None

Principal Investigator: W. A. Loeven (E.O.D. 10/11/67)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	.83
Professional:	.33
Others:	.5

Project Description:

Objectives: Elastic tissue has been studied by chemical analysis and enzymatically by following the elastolysis of aorta powder, nuchal ligament or isolated elastin incubated with the enzymes of the elastase complex.

Studies in the last few years have shown that the following enzymes belong to this complex:

a. Elastoproteinase (or elastase itself), the proteolytic component of this complex which dissolves the elastin protein.

b. Elastomucases EM-I and Em-S (also called elastomucoproteinase) which primarily attack the carbohydrate moieties present in elastic tissue and even in isolated elastin;

c. Elastolipoproteinase, the lipolytic component which releases the lipid moieties.

On the basis of the supposition that the structure of elastic tissue can only be maintained by a complex balance of these elastolytic enzymes held in check by their individual inhibitors

(also isolated from or in any case identified in blood and pancreas) and that a disturbance in this balance may intervene in the aging process and in diseases affecting elastic tissue and elastin. This relationship has been studied by various workers.

Especially the results of the study concerning the influence of age on the enzyme content in the pancreas and of the elastoproteinase inhibitor in blood, however, have failed to provide clear verdicts. This is in the first place due to the fact that in the older investigations very little was known about the complexity of the enzyme "elastase" and secondly, it was unknown that the enzyme elastoproteinase itself is also present in the circulation (in this way only the excess of enzyme inhibitor was measured).

The present author has described the influence of age on the content of elastomucase and elastoproteinase in the pancreas of necropsied individuals obtained from the Pathology Laboratory of the Leiden University in Holland. Especially in males, a very pronounced age relationship was found. In females, however, this effect of age was much less pronounced and the hypothesis was put forward that hormonal regulation mechanisms may also play a role in the enzyme production or secretion. The menopause seems to be a factor in these processes. Nearly the same changes with age take place in human blood. Still running experiments in Leiden in cooperation with the Department of Cardiovascular Diseases already permit the conclusion that the enzyme and inhibitor concentrations in the blood not only vary with age but that the lipoprotein content in the circulation is also an important factor (especially the content of the β-lipoproteins).

Here too, the data obtained from the females differ from those of the males.

The objective of this study is to determine whether or not the same changes in enzyme and inhibitor concentrations can be observed in the rat during growth and aging. The rat is chosen as the experimental animal because here we can measure enzyme and inhibitor concentrations in both the pancreas and the blood. Measurements were impossible to perform in the human.

Methods Employed: Pancreatic tissue will be removed, homogenized, extracted, dialyzed and finally liophylized. The final dried extract will be used for measurements of enzyme and inhibitor content: a. content of elastoproteinase; b. content of elastomucase; c. content of the elastoproteinase inhibitor.

Due to this program it will be necessary to collect pancreata from more than one rat to perform these measurements.

From the young rats (up to 6 months) pancreata of five animals of the same age groups must be taken together as one sample; from the older rats three of the same age groups are enough to form one sample.

This means that for a statistical evaluation to be meaningful 25 rats in the young age groups and 15 rats in the older age groups are necessary. The age groups will cover 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, and 24 month old rats, both males and females. The total amounts of animals are 240 males and 40 females.

The investigator will spend 50% of his time on this project. A technician will be needed full time (100% of his time) to assist in this project.

Major Findings: Since only very small amounts of enzyme and inhibitor can be obtained from these rats pancreata, a separation of elastoproteinase and elastomucase by means of column chromatography is impossible. In the preceding months, however, we have found that by using three different kinds of elastin as substrate it is still possible to measure separately the elastoproteinase and elastomucase contents in the pancreatic extracts, even in the presence of the elastoproteinase inhibitor. The method of Hall (Biochem. J. 101, 29, 1966) with CongoRed-stained elastin as substrate enables us to measure the elastoproteinase without the interfering effect of his inhibitor. At the same time it was observed that lowering of the ionic strength of the buffer solution results in a higher enzyme activity.

The results obtained with orcein-stained elastin as substrate were very unexpected and also solved the question which exists for many years why none of the American investigators who studied elastin and the enzymes of the elastase complex are able to confirm the results of Banga and Balo (Budapest), Hall and co-workers (Leeds) and myself concerning the enzyme elastomucase and also deny or ignore its existence. With orcein -stained elastin as substrate it is impossible to measure the activity of elastomucase, presumably due to the fact that the orcein blocks some of the groups in the elastin-carbohydrate complex essential for the enzymic reaction of elastomucase. With this substrate the activity of elastoproteinase can be measured without the interfering effect of the elastomucase. These findings will be published soon and methods have been worked out for routine determination of these enzymes in the rat pancreatic extracts.

Finally, it was found that the extraction technique routinely used for the extraction of crude enzyme samples from commercially available pancreas powder appeared to give less results with the rat pancreata. This method was also slightly modified (e.g., extraction of the pancreas with a carbonate buffer pH 8.8 instead of the acetate buffer of pH 4.7).

Proposed Course of Project: Since this project has been started very recently, the enzyme content in the pancreata of male and female rats will be measured with the methods described above in the sections Methods Employed and Major Findings from preliminary work.

Honors and Awards: None

Publications: None

NICHD ANNUAL REPORT
July 1, 1967, through June 30, 1968
DIRECT OPERATIONS

OFFICE OF DIRECTOR

CONTRACT AND COLLABORATIVE RESEARCH

Summary. XVI
(see Director's Summary)

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Office of Program Planning and Evaluation
Contract and Collaborative Research

Contract Title: Pediatric Manpower Training Project
(Resources Inventory)
Contractor: Board of Regents of the University of Washington, Seattle, Washington. Ralph J. Wedgwood, M.D.
Money Allocated: $53,890.00

Objectives: This study will extend the analysis of data already collected as part of the University of Washington's Pediatric Manpower Survey Project which was funded by the Children's Bureau and produced the document, "An Inventory of Pediatric Departments." The current contract will further analyze the on-hand data to produce six additional reports as listed below:

1. The Funding of Pediatric Departments
2. Faculty of Pediatric Departments
3. Clinical and Research Resources in Pediatric Departments
4. Teaching and Training in Pediatric Departments
5. Determinants of Strength in Pediatric Departments
6. The Perceived Needs and Future Plans of Pediatric Departments

Significance to Biomedical Research and Program of the Institute: The reports resulting from this contract may be used to assess the present status of manpower training in child health and for program planning for future training needs on a national scale.

Proposed Course: This contract was initiated December 28, 1967 and is expected to expire on December 27, 1968.

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July 1, 1967 through June 30, 1968

Epidemiology and Biometry Branch
Contract and Collaborative Research

Contract Title : Study of Correlates of Intrauterine Contraceptive Device Effectiveness
Contractor : University of California, Berkeley School of Public Health
Money Allocated: $192,237

Objectives: The aim of the study is to search for physiological, attitudinal and demographic factors which are associated with effectiveness and acceptability of the IUD in low motivated groups. The approach is to obtain information, through standardized procedures on women provided with IUD's at public family planning clinics throughout California.

Summary: Information has been collected on 8,475 women accepting IUD's at 11 county health department and hospital clinics from July 1964 through November 1967. Study activity during the past year has been directed toward completing the coding and keypunching of a large backlog of follow-up record information, completing a pilot study to evaluate methods and ascertain costs of locating and obtaining IUD information on women who do not return for clinic follow-up and developing computer programs to provide analysis of initial and follow-up record data. Editing, coding and computerized consistency check systems have been developed. Preliminary analysis of data from a special study of the effectiveness of different sizes and types of IUD's has been carried out.

Significance to Biomedical Research and Program of the Institute: This is an area of study of increasing importance as more IUD's are used and about which there is much to learn.

Proposed Course: This Contract is scheduled for four years. The second year runs from June 26, 1968 through June 25, 1969, and has been approved for renewal at $143,256. The schedule for the coming year will be to terminate remaining patient groups, continue special follow-up efforts on hard-to-find women, and begin more complex analyses.

NICHD ANNUAL REPORT
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Population Research Branch

Contract and Collaborative Research

A. Reproductive biology.

Population research in the biological sciences is directed toward means to fertility regulation. The acquisition of basic information concerning reproductive processes is the first phase in research in contraceptive technology. Reproductive biology is a well established research field receiving the attention of a number of distinguished investigators and has been an integral part of the Institute's extramural and intramural programs. There is still, however, a great deal of information of a fundamental nature which must be obtained in order to proceed to new contraceptive techniques. An analysis of the field by a subcommittee of the Population Research Advisory Committee which held a series of meetings in FY 1968 identified several topics in the field of reproductive biology in which it was thought further research will most likely lead to the subsequent development of new methods of fertility control.

An example of a topic with such potential pay-off which is being considered is the study of the corpus luteum. In certain species there appears to be a luteolytic agent produced by the uterus, which if administered at the proper time will cause the corpus luteum to regress as if fertilization had not occurred. The goal of a concerted study of corpus luteum function would be to develop a medication which could be taken by a woman at the time of her expected menses. The drug would inhibit the action of the corpus luteum in a physiologic manner and cause menstruation to occur at the expected time. If such an agent were identified, NICHD would be prepared to negotiate contracts to conduct drug testing and clinical trials.

Other examples of potential significant advances in contraceptive technology include studies of sperm capacitation and the composition of seminal fluid, leading to improved male contraceptives, and studies of the local events at ovulation, leading to efficient methods of periodic abstinence. Still another involves the employment of bioengineering with the intent to develop simple and effective means to produce permanent sterilization.

An organized program of research of this type will help satisfy some of

the major requirements of the population research field. It will make possible the collection of fundamental information which is essential to significant advances. It will support scientists already in the field and, by directing investigators in other fields to a subject of great social importance, will give population research the stimulus of interdisciplinary action it so urgently needs. Furthermore, its collaborative nature will provide some measure of stable institutional support to several population research centers.

Only one contract supported research in reproductive biology in FY 1968. This was for a symposium held at Washington State University on the mammalian oviduct, a subject of special interest to the Institute since remarkably little is known about the anatomy and physiology of the structure in which important early events in reproduction take place. Proceedings of the symposium are being published.

Because of the need to develop a variety of new contraceptive techniques which must be based on a clearer understanding of certain phases of the reproductive cycle, it is planned to support some basic studies in threshold areas, such as those mentioned above, in the contract program in FY 1969.

B. Contraceptive methodology.

This is the second phase of the research program and is based to a large extent upon fundamental findings in reproductive biology. The goals are the development of new methods and the assurance that all methods in use are safe. Contracts in FY 1968 have been chiefly in this area, supporting research on the mechanism of action of contraceptives, particularly oral steroids.

In FY 1968 the largest of the contracts supported by NICHD evaluating the effects of oral contraceptives was negotiated with the Kaiser Foundation Research Institute. The purpose of the project is to undertake a long-term study of a variety of medical effects of oral contraceptives in women. The study is being conducted at Walnut Creek Hospital in California and involves special examinations of about 20,000 women, half of whom are expected to be on oral contraceptives and half using other methods of contraception. The examinations employ the multiphasic screening technique and are designed to detect both biological and behavioral indicators of problems associated with the use of contraceptives. Some data will be available by the end of this year, but information on the long-term effects of oral contraceptives, such as the possible relationship between their use and carcinoma of the breast or cervix, will require several years.

Other contracts to study oral contraceptives are of a somewhat more restricted nature but deal with subjects of great importance. One is designed to see if there is a relationship between the use of oral contraceptives and the progression of cervical dysplasia, a pathological condition which can lead to carcinoma of the cervix. This project will yield information within two or three years. Another contract is with the University of London and is devoted to the study of relationships between

oral contraceptives and glucose and lipid metabolism. A number of studies in recent months have shown that these agents produce alterations in these and allied substances. The study is designed to measure these changes and to evaluate their significance.

There is considerable interest by both public and private agencies in the mechanism of action and medical effects of intrauterine devices. In FY 1968 the Institute continued support begun in 1964 of reproductive physiologists in the Department of Agriculture who have been studying the effects of intrauterine foreign bodies on the reproductive performance of several experimental animals. This work is of importance since the mechanism of actions of IUD's is not well understood. The Institute also wrote a contract in 1967 with pharmacologists at the Institute of Postgraduate Medical Education and Research in India to see if IUD's have an effect on oxytocin levels in humans. It is generally accepted that IUD's work locally and have little systemic effect. Recent work, however, suggests that there may be a systemic effect manifested by an alteration of oxytocin levels. It is the purpose of this contract to clarify this issue.

C. Family planning and population planning.

This is the least well-developed area of population research and involves individual, interactional, familial, institutional, and societal processes involved in decisions about family planning and the effective implementation of population control programs and their consequences.

Currently only two contracts in this area are being funded: one is the continuation of a survey of American family planning practices conducted by Princeton University, and the other is for the continued support of the training and experience of physicians in the epidemiology of fertility regulation through the National Communicable Disease Center in Atlanta.

Since the NICHD has been designated the focal point in NIH for research in population control and family planning programs and additional funds are being allocated for contract support in this area, the population research program will be expanded to include more contracts in the behavioral and social sciences. It is planned to develop a contract instituting a recurring family growth survey whose primary focus will be knowledge, attitudes and practices related to procreation and contraception. This will enhance both demographic and family planning knowledge. Another area for research is the determinants and consequences of fertility patterns and the origins and effects of national population policies.

Additionally a great deal must be learned about the psychological and social determinants and effects of contraceptive practices on parents, children, family relationships, sex behavior, and family stability. It has been argued that the effective use of contraception may be one of the most powerful tools for promoting mental health, since it permits individuals to make effective and responsible decisions about an important part of their lives by removing sexuality from reproduction. These decisions warrant study. Abortion is similarly heavily laden with social and legal problems and is a legitimate subject for study. There are no good data,

for instance, on the number of criminal abortions performed each year in the United States. Estimates range from 200,000 to one million. Also the liberalization of the laws for legal abortion which is occurring in several states, provides a unique opportunity to study the effects of innovation on attitudes and practices.

D. Reproduction Information Center.

This center, administratively included under the Scientific Information Centers Branch, will produce two reports in FY 68. Under a contract with the Scientific Information Exchange it is preparing a survey of the support of research in reproduction and family planning for 1966. It is also preparing a comprehensive compilation of gonadotropin literature published in 1966. The center has also started to collect information for a directory of investigators working in reproduction in various countries and regions including India and Latin America. In addition, the center is negotiating with the National Library of Medicine the preparation of a citation journal covering the literature in population research. This will include reproductive biology up to implantation, the development and evaluation of contraceptives, and psychological and social sciences as they relate to population research.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Branch
Contract and Collaborative Research

Contract Title: To Survey the Fertility and Contraceptive Practices of the American Population
Contractor: Princeton University
Money Allocated: None needed in FY 1968 (contract extended without additional funds)

Objectives: The objective of the contract is to complete the analysis of a survey of family planning practices among American families which was made in 1965. This survey will have continuity with the first two "Growth of American Family (GAF)" studies which were conducted in 1955 and 1960, so as to record the rapidly changing conditions of the following five years and fix the appearance of new trends.

The field work phase of the study has been completed. This included the collection of data and the editing, coding, and card punching. The primary objective of the present contract was to complete this analysis and provide the Institute and the scientific community with the results. As the data is collected the contractor will continue to analyze it and individual articles will be prepared as the various analyses are completed.

Of significance this year was an analysis of the drop-out rate for women on oral contraception. About one-third of all women who had used the pill at any time since 1960 had discontinued use (either permanently or temporarily) by the time of interview in 1965. The majority of these women stopped because of what they perceived as unpleasant side effects of the drug, most of which related either to undesirable reactions commonly associated with pregnancy or to problems connected with the menstrual cycle. The proportion continuing for at least one year is now approximately 80%. Women under 30 years of age experienced a lower drop-out rate than older women and women with three or more children showed a higher drop-out rate than women with fewer children. There was also a consistent tendency for women with the least education to experience the highest drop-out rates. And finally, women who discontinue use of oral contraception tend mainly to return to the method they used before the pill, although there is some tendency to use other new methods such as foam and the IUD.

Significance to Biomedical Research and Program of the Institute: This study will serve as a base of reference for prospective studies on contraceptive practices and their influence upon the health of women and children. It is an essential part of the public health aspect of the study of contraceptives.

Proposed Course: The original contract was initiated June 29, 1965. The present contract extended the period of study from June 28, 1968 to August 31, 1969 without additional funds.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Program
Contract and Collaborative Research

Contract Title: Symposium on the Mammalian Oviduct Reproduction Research Techniques
Contractor: Washington State University
Money Allocated: $4,750

Objectives: The purpose of the 5-day symposium held at Washington State University (July 21-August 4, 1967) was to provide interchange of information and encourage established investigators in biological and health services to give more attention to the function of the oviduct, a neglected organ in reproduction research. The additional fund allocation this year was to provide color plates and electromicrographs for the symposium proceedings which are to be published by the University of Chicago Press at no charge to the Government or the contractor.

Significance to Biomedical Research and Program of the Institute: This symposium is of direct interest to this Institute and one which can be carried out by the University for the benefit of NICHD.

Proposed Course: The contract was initiated March 1, 1967 and will terminate June 30, 1968.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Program
Contract and Collaborative Research

Contract Title: Study of Correlates of Intrauterine Contraceptive Device Effectiveness
Contractor: University of California, Berkeley
Money Allocated: Approximately $170,000 (not yet funded)

Objectives: The purpose of this contract is to study the various correlates of intrauterine device effectiveness, including physiological, attitudinal, and demographic factors which may relate to the effectiveness of the device in a sample of 8,000 public clinic patients in California representing a low-motivated, low socioeconomic group needing effective contraception and generally lacking the desire for more children. Study activity during the past year has been directed toward completing the coding and keypunching of a large back-log of follow-up record information, completing a pilot study to evaluate methods and ascertain costs of locating and obtaining IUD information on women who do not return for clinic follow-up and developing computer programs to provide analysis of initial and follow-up record data. Editing, coding and computerized consistency check systems have been developed. Preliminary analysis of data from a special study of the effectiveness of different sizes and types of IUD's has been carried out. Intake of new patients was terminated at all clinics by January 15, 1968 and the immediate post-partum insertions at the last clinic to admit new patients will continue through 1968. Post-partum insertion records have been punched through July 1967.

Significance to Biomedical Research and Program of the Institute: This is an area of study of increasing importance as the use of IUD's has increased greatly through out the world and the correlates of IUD effectiveness must be assessed.

Proposed Course: The Institute has approved an extension to the present contract of an additional year through June 25, 1969. This extension is currently being negotiated by the Contract Research Section, SMB. It is expected that it will take three additional years, until 1971, to complete the study.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Branch
Contract and Collaborative Research

Contract Title: Effects of Steroid Contraception on Cervical Dysplasia
Contractor: University of California, Los Angeles
Money Allocated: $90,000 (not yet funded)

Objectives: The contract is to support the study of the cumulative effects of steroid contraception on the occurrence of cervical cancer by studying dysplasia of the cervix. The study is designed so that probability sampling from a large base population will modify some of the difficulties inherent in longitudinal studies. Of 290 patients eligible for the study as of February 1, 1968 and screened by Papanicolaou smears, 34 cases of dysplasia had been identified and entered the follow-up program. A systematic smaple of women with normal Papanicolaou smears had also been selected and entered the follow-up program. The dysplasia patients were characterized as follows: 20 had been on pills; 14 had IUD's; 24 were between the ages 15 and 24; 28 were Negro; and 12 had had one delivery.

Significance to Biomedical Research and Program of the Institute: This is a study subject of significant priority to the medical community and to the program of this Institute.

Proposed Course: This contract was initiated June 23, 1967 to extend through June 22, 1968. The extension of this contract for another year has been approved by this Institute and is currently being negotiated by the Research Contract Section, SMB. It is anticipated that the study will require a total of five years of support.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Program
Contract and Collaborative Research

Contract Title: Contraceptive Surveillance Studies
Contractor: Kaiser Foundation
Money Allocated: None needed in FY 1968

Objectives: The primary objectives of the contract will be to evaluate all of the clinical differences between users and non-users of contraceptive drugs and to develop a system for continuing surveillance over a long period of time.

Although this immediate study design is directed to evaluating oral contraceptive drugs, this system of surveillance will eventually have the flexibility (a) to study any contraceptive agents, (b) to incorporate improved evaluative methods, and (c) to permit "spin-off" or special side studies to investigate in greater depth particular areas of interest.

Significance to Biomedical Research and Program of the Institute: This contractor is in the unique position of possessing all of the capabilities necessary to the successful conduct of an epidemiological study desired by this Institute. It has a study population located in a single geographic area utilizing single medical facilities, resulting in a self-contained medical community. The contractor also has the clinical and laboratory staff and facilities to conduct this study. The staff is research oriented and experienced in the automatic screening and evaluation system which is available for this study.

Proposed Course: This contract is in its initial contract year which began June 26, 1967. It is expected to continue for at least five years, subject to periodic review and negotiation.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Program
Contract and Collaborative Research

Contract Title:	Effects of Oral Contraceptives on Glucose and Lipid Metabolism
Contractor:	University of London, England
Money Allocated:	Approximately $43,000 (not yet funded)

Objectives: The contract is to support a study of the metabolic effects induced by oral contraceptives and the possible long-term effects oral contraceptives may have on metabolic processes.

Significance to Biomedical Research and Program of the Institute: Preliminary results of this study confirm that oral contraceptive therapy is associated with impaired oral glucose tolerance even though fasting plasma glucose levels are normal. In addition, fasting blood pyruvate levels are abnormally high and their increase after oral or intravenous glucose may also be abnormally high. Many more subjects studied longitudinally will be required to discover which factors, such as obesity, family history of diabetes, type of oral contraceptive and duration of therapy, are associated with the various changes of carbohydrate, lipid and intermediary metabolism. It is already clear, however, that certain changes during oral contraceptive administration are extremely common and it is this Institute's concern to seek answers to the questions raised.

Proposed Course: This contract was initiated in June 1, 1967 and an extension for its second year has been approved by the Institute and is being negotiated by the Contract Research Branch, SMB. It is estimated that the study will take another six years (1974).

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Program
Contract and Collaborative Research

Contract Title: Effects of IUD on Oxytocin Level
Contractor: Institute of Postgraduate Medical Education and Research
Chandigarh, India
Money Allocated: $17,750

Objectives: This contract is to support investigation between oxytocin and the intrauterine device in experimental animals and in humans in the hope of finding a solution to the many problems that have arisen with the use of the device, such as bleeding, pain, expulsion, and the need to find out as soon as possible the mechanism of action of the device.

Significance to Biomedical Research and Program of the Institute: Because of the widespread use of the IUD throughout the world and the lack of understanding of its mechanism of action the Institute has placed high priority on this research. It is urgent that studies such as this be conducted.

Proposed Course: The contract was initiated August 18, 1967 and was extended an additional year through August 17, 1969 with no additional funds.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Program
Contract and Collaborative Research

Contract Title: Support of the Committee on Population
Contractor: National Academy of Sciences
Money Allocated: $45,000

Objectives: The purpose of this contract is to provide support for the Committee on Population of the National Academy of Sciences which will prepare and publish in collaboration with the British Royal Society's Population Study Group a report, with specific recommendations for action programs in the area of Population and Family Planning. The report will include the following areas:

1. Improvement in contraceptive technology.
2. Improvement in methods of family planning services.
3. Economic aspects of population control.
4. Standardization of surveys.

Significance to Biomedical Research and Program of the Institute: A study of the sort outlined above coincides directly with program interests of this Institute and will be of immediate and practical assistance to the Institute as it develops its programs in population research.

Proposed Course: This contract was initiated on August 1, 1967 and will run until January 31, 1969.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Program
Contract and Collaborative Research

Contract Title: Physiological Mechanisms involved in Reactions to Implanted Devices in Animal Uteri. Psychological and Physiological Effects of Oviduct Blockage in Animals.

Contractor: Agriculture Research Service, U.S. Department of Agriculture (reimbursable agreement)

Money Allocated: $82,000

Objectives: Research work conducted during the past three years has provided considerable information on the diverse and varying physiological effects of intrauterine devices in various animal species. Further study will cover effects of IUD on ovulation and corpus luteum development, on spermicidal effects in the uterus, on uterine vascular function and the chemistry of endometrial ground substance, on uterine motility and on sexual behavior. The work will be done with sheep, cattle, swine, rabbits and guinea pigs.

Significance to Biomedical Research and Program of the Institute: Because of the widespread use of intrauterine devices by women throughout the world and the incomplete knowledge of how they work in preventing conception, or the physiological and psychological effects they may have on these women, this research and its implications are extremely important.

Proposed Course: This Institute has been supporting this research by reimbursable agreement with the Department of Agriculture since January 1965 and has renewed the contract annually since then. The current agreement extends through June 30, 1968.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Program
Contract and Collaborative Research

Contract Title: Indian Service Cooperative IUCD Study
Contractor: U.S. Public Health Service Indian Hospital
Gallup, New Mexico (reimbursable agreement)
Money Allocated: $1,250

Objectives: The specific aim of this contract was to support partially the expansion and continuation of the Indian Service Study of Intrauterine Contraceptive Devices. This study was designed to demonstrate the feasibility of general practitioners using such a method of contraception, to gather and examine histologically more uteri from women using intrauterine contraceptive devices, and to evaluate the effectiveness and safety of this method in an indigent, largely illiterate population with an inadequate knowledge of modern health practices who live remote from medical facilities.

Significance to Biomedical Research and Program of the Institute: This is an important study on intrauterine contraception in a unique population group. The information is being assembled for use by the National Committee on Maternal Health so that meaningful comparisons with the large cooperative study of the NCMH are possible.

Proposed Course: The contract was initiated January 1, 1967 and terminated on December 31, 1967.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Program
Contract and Collaborative Research

Contract Title: To support training and experience in the relationship of epidemiology to fertility regulations.
Contractor: U.S. Public Health Service Communicable Disease Center, Atlanta, Georgia (reimbursable agreement)
Money Allocated: $114,000

Objectives: This program supports six epidemiological intelligence service officers who are assigned to family planning program evaluation research. These officers receive their basic training and experience in the field under the jurisdiction of the Communicable Disease Center.

Significance to Biomedical Research and Program of the Institute: Because of the significant number of women currently taking oral contraceptives and participating in family planning programs today, a sound epidemiologic approach must be developed to evaluate the effectiveness of these programs. This is of immediate interest to this Institute and to the scientific community.

Proposed Course: The agreement was initiated July 1, 1966 to run for one year. It has continued in FY 68 and is expected to continue and expand again in FY 69.

Population Research Program
Contract and Collaborative Research

Contract Title: Conference on The Metabolic Effects of Gonadal Hormones and Contraceptive Steroids
Contractor: Harvard University, School of Public Health
Money Allocated: $30,000 (not yet funded)

Objectives: The purpose of this contract is to support a conference which will be held in the fall of 1968. The conference will appraise the current state of knowledge concerning the actions of steroid substances on the metabolic functions of humans. There will be an attempt to collect current work in relation to general knowledge of the field. The areas needing further emphasis will also be explored.

Significance to Biomedical Research and Program of the Institute: At present approximately ten million women are taking oral contraceptives whose long-term effects are not yet known. Two ways may be used to gain this information: 1) by epidemiological type studies of large populations and 2) by intensive study of the biological effects on the human organism by established procedures. This latter appears to be a necessary and neglected area and perhaps the most fruitful approach currently available. Support of the conference is therefore of direct interest to NICHD.

Proposed Course: This Institute has approved a one-year contract for this conference which is currently being negotiated by the Contract Research Section, SMB.

NICHD ANNUAL REPORT
July 1, 1967 through June 30, 1968

Population Research Program
Contract and Collaborative Research

Contract Title: The Development of Measures of Unwanted Childbearing
Contractor: DHEW, National Center for Health Statistics (reimbursable agreement)
Money Allocated: $71,000 (not yet funded)

Objectives: The purpose of this contract is to aid in the development of questions that can be used in interview and mail surveys that will be conducted by the Center to measure the extent and degree of uncontrolled fertility in the United States. This study should also yield considerable practical experience on the conduct of surveys on the family planning practices and attitudes of women who have high fertility and low educational attainment.

Significance to Biomedical Research and Program of the Institute: Insofar as the core of "excess fertility" problem is concentrated among such women this experience will aid greatly in the development of nationwide family planning surveys that the NCHS plans to conduct in the future. This clearly is of direct concern to the Population Research Program of NICHD.

Proposed Course: This reimbursable agreement is expected to extend through FY 1969. The Institute has agreed to cover the costs incurred by the NCHS this year (FY 1968) and the agreement is currently being negotiated.

1. Developmental Biology Branch
2. Reproduction Anthropometry Section
3. NIH, Bethesda, Md.

PHS - NIH
Individual Contract Report
July 1, 1967 through June 30, 1968

Contract Title: Statistical studies of menstruation and reproduction among indigenous peoples and Caucasians in Alaska

Contractor: Arctic Health Research Laboratory (AHRL)

Amount Allocated: $40,100 in additional funds under Reimbursable Agreement for a second year of operations

Project Description:

Objectives:

(a) To establish panels of menstrual history record keepers among the Eskimos and Caucasians now resident in Alaska.

(b) To provide supervision of such record keeping activities among female members of the indigenous communities, and to give them instruction and other assistance toward achieving desirable standards of related personal hygiene.

(c) To increase the knowledge of age at menarche among girls attending residence and village schools of the Bureau of Indian Affairs in Alaska, using a census approach.

Accomplishments:

Despite the disturbances to all activities occasioned by physical transfer of AHRL headquarters from Anchorage to Fairbanks last summer, the resulting loss of staff without prompt replacement in certain crucial appointments, and the massive flooding of a large are of Fairbanks a few months later, the work of the public health nurses implementing this program was maintained without serious loss. A field station for epidemiological research, established in Anchorage, served in part as a base of operations for the research program.

Residents of nine Eskimo villages in the Yukon-Kuskokwin area in addition to the town of Bethel, now comprise the study panel. A Northern Eskimo panel was developed at Pt. Barrow last Fall, adding 220 cases to yield a total Eskimo enrollment of nearly one thousand persons. Enrollments in the Eskimo panel were closed as of the end of last March except for addition of menarche cases as they occur.

Planned checks of the Eskimo MH calendars every month by the field nurses was found unnecessary, only a few failing to keep their records without such review. This good fortune made visits delayed to the second or third month, when imposed by bad flying weather, free of penalty on the program. Very few Eskimos are failing to keep their records in accord with instructions. Partial review of these monthly calendars, which are collected periodically by the nurses, at present seems to indicate any difference in menstrual cyclicity between the Eskimo records and those of the large Minnesota series of Caucasians is not detectable without the statistical analysis which is planned to follow later.

Since the Eskimo village and town studies would yield relatively few menarche cases, the supplementary survey of girls in selected schools of the Bureau of Indian Affairs was undertaken. This census-type inquiry aims basically to count the pre-menarche and post-menarche cases at each year of age, adding whatever detail about age at menarche that can be obtained from those who have entered the menstrual years. Probit-type analyses of the information will permit estimation of the median age at menarche, by racial groupings (Eskimo, Aleut, and Indian), to augment the currently recorded data on age at menarche in the villages. Some 660 records of menarche have been obtained in this survey from three boarding schools and eight village schools. These data are now being coded in this office for input to computer and will be analysed at the University of Minnesota under contractual agreement.

A panel of Caucasian record keepers, established early in 1967 through mailed solicitation of over 900 female teachers and certain administrative staff of the Anchorage Borough School District, yielded less than 200 active record keepers. A second solicitation this past winter, stressing interest in records involving control with oral contraceptives, is yielding some additional panel members. It is expected, however, that this cohort will remain relatively small. Returns received for calendar year 1967 show these records to be very well kept, differing little (if any) in cyclicity characteristics from those of the Minnesota series. A new coding protocol is under development for this series. It is anticipated that this protocol will serve as a model for revision of the MS master tape file, enabling more efficient computer operations thereafter on all records maintained on a calendar year basis.

1. Developmental Biology Branch
2. Reproduction Anthropometry Section
3. NIH, Bethesda, Md.

PHS - NIH
Individual Contract Report
July 1, 1966 through June 30, 1967

Contract Title: Quantitative Definition of the Temporal Characteristics of the Human Menstrual Cycle

Contractor: Regents of the University of Minnesota, on behalf of staffs of
(a) the School of Public Health, Biometry Division, and
(b) the University Health Service

Amount Allocated: $27,760 in additional funds for a third year of operations

Project Description:

Objectives:

To provide continued participation of the University in the menstrual history (MH) program, initiated and in part continuously maintained by it since 1934, through provision of computer and allied services for (a) the storage of information, and (b) preliminary statistical analysis of data assembled under the total MH program as now enlarged with NIH support.

Accomplishments:

Throughout FY '68 the University Health Service has continued to contribute space, the Biometry Division has provided staff and ancillary facilities, and the Medical School has contributed improved computer facilities. Since this contract involves close collaborative work with staff of the Section in all aspects of the creation of a master file of validated MH data on magnetic tape, and in the design of arrangements for computer output of listings and statistical tabulations and computations, descriptive statement of these facets of the contract operations has been included in the foregoing MH Project Report. The additional information given below is confined to a numerical summary of production under the contract as of 1 May 1968.

(a) Successive computer runs with required modifications of an elaborately formulated consistency checking program have resulted in final acceptance of a master tape (magnetic) storing all MH information as received and coded by us from initiation of the program in 1934 through calendar year 1967.

(b) Twelve analysis programs were written and tested on a representative sample of 200 case histories, ten of them being debugged and cleared for final application to the master tape while the consistency checking operations were progressing. The final runs on the master tape for the completed programs have now been made and the printouts delivered to the Section in the formats requested.

(c) The two additional computer outputs will be available to the Section in the form requested before close of this fiscal year. Additional requests are in formulation by this Section and good progress in writing the necessary programs before 30 June is anticipated.

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Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

The Perinatal Biology and Infant Mortality Branch is supporting through the contract mechanism, four projects dealing with (1) The Role of Viral Infection in Respiratory Sudden Death in Infants, (2) Standardization of the African Pygmy Goat as a Laboratory Animal, (3) Diagnosis and Treatment of Disorders Affecting the Intrauterine Patient, and (4) Study of Selected Aspects of the Psychodynamics of Pregnancy.

The National Institute of Child Health and Human Development has contracted with the Children's Hospital Research Foundation, Washington, D.C., for a study of unexpected infant death in the Washington, D.C. area, with particular emphasis on upper respiratory viral infection as a possible cause of sudden infant death. The investigation begins as soon as possible after the death of the infant and includes not only cultures of the body fluids of infants, but also sputum and serum cultures from the family members, for indications of viral infection. Some epidemiologic information relative to the occurrence of sudden unexpected infant death is also obtained.

Dr. James Metcalfe, University of Oregon Medical School, is now in his second year of an NICHD contract to develop a Pygmy goat colony as a resource for research purposes. It is hoped that the Pygmy goat (which is easy to handle, economical to feed and house, easily trained, has a long breeding season, and reaches maturity at six months) will prove to be a preferred alternative to the sheep for fundamental research relative to the physiology of fetal growth and development and neonatal adaptation. The increasing interest in the physiology of the fetal-maternal complex demands the development of an experimental animal with uniform physical and genetic characteristics; it appears that the Pygmy goat may well fulfill these requirements.

The document concerned with the diagnosis and treatment of disorders affecting the intrauterine patient, which will result from our contract with Columbia University, is anticipated to serve as a stimulus for the development of a broad research program related to reducing perinatal and infant morbidity and mortality. This document will contain information relevant to the present state of knowledge in the area of prenatal disorders, the identification of gap areas where new diagnostic methods are needed to detect deviations from normal intrauterine fetal development, and an indication of directions for future research based on the application of already extensive knowledge and methods.

Under NICHD contract funds, the Group Health Association, Inc., Washington, D.C., is preparing a scientific report on specific aspects of the psychodynamics of pregnancy. A major gap in the area of perinatal and infant survival and well being concerns psycho-social factors of pregnancy and their relationships to pregnancy outcome and perinatal and infant mortality. It is anticipated that this report, based on the analysis of data from the Association's

Demonstration Project on Prenatal and Early Postnatal Adaptation, will reveal new knowledge on the psychological adaptation to pregnancy and its relationship to pregnancy outcome and subsequent maternal-infant adaptation.

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Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : The Role of Viral Infection in Respiratory Sudden Death in Infants PH 43-66-917
Contractor : Children's Hospital Research Foundation (Dr. James R. Patrick)
2125 13th Street, N.W.
Washington, D. C. 20009
Money Allocated: $100,000

Objectives: To study as soon as possible after death, each case of unexpected death in infancy which occurs in Washington, D.C. The child is cultured for viral agents, mycoplasma and bacteria. Family members in the child's household are cultured for viruses. Serum samples are taken from the child examined for antibodies as an indication of viral infection. Acute and convalescent serum are taken from family members and antibody titers are studied as an index of viral infection. Serum and tissues from the child are banked for further studies in the future.

Methods Employed: With the cooperation of the coroner of the District of Columbia, all cases of Sudden Death in Infancy are reported to Dr. Patrick's group at Children's Hospital. Postmortem examinations, as required by law, are performed within three to four hours of the time of discovery of the body. Anal and pharyngeal swabs are taken for viral and mycoplasma isolation. Heart blood and tissue specimens are taken sterilly for bacteriological examination. A team of physician and nurse visit the child's home, and obtain blood specimens from family members for antibody studies and pharyngeal and oral swabs for viral study. They learn the circumstances surrounding the child's death. Three weeks later they obtain a second set of blood specimens from members of the family.

Results: Case collection started on October 31, 1966. Thus far, 58 children have died of Sudden Death in Infancy in the District of Columbia. In most cases post mortem examinations have been performed within 3-4 hours of the time of discovery of the body. At autopsy, adequate explanations were found for nineteen of these deaths, leaving thirty-nine unexplained. Demonstrated virus infections occurred in the unexplained dead and their surviving young household contacts at rates approaching those seen in infants and children hospitalized with respiratory disease; in all, fifty-six percent of the unexplained dead yielded one or more viruses and/or pathogenic bacteria. Evidence obtained to date indicates that there is no direct cause and effect relationship between sudden unexplained infant death, mycoplasma, and pleuropneumonialike organisms (PPLO). However, a high incidence of upper respiratory viral infection has been found in victims of crib death and in their families.

Significance to Biomedical Research and Program of the Institute: The causes of sudden death in infancy are unknown. Twenty-five to fifty thousand children mostly in the two month old to six month old age range, are thought to die each year with the findings of this syndrome. Viral infection has frequently been postulated as a cause of these deaths. It is hoped that this study will clarify that point. By performing postmortem studies soon after death, it is hoped that less hardy organisms, which might die soon after the child's death, may be isolated.

Proposed Course: It is planned to expand on the concept of expected viral prevalence in the ill, the well, and the "representative" child with reference to the sudden death case and its household contacts. Data from longitudinally followed respiratory virus vaccines will be developed to supplement data from the respiratory illness program. With these data, we should be able to establish better norms for illness, virus isolations, and seroconversions particularly as they vary in this community with age of the child and by month of the year. These norms should then better enable us to compare the rates of infection in the community with those of the unexplained deaths and their households.

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Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Standardization of the African Pygmy Goat as a Laboratory Animal PH-43-67-688
Contractor : University of Oregon Medical School
Money Allocated: $45,194

Objectives: The contract with Dr. James Metcalfe, University of Oregon Medical School, for the development of a Pygmy goat colony as a resource for research purposes has been negotiated for a second year. Development of this resource will make possible studies in which the growth, developmental, and genetic characteristics of these animals will be measured with a view to evaluating the results of artificial insemination, semen preservation, and ova transplantation in this species, as well as various studies related to perinatal biology, fetal growth and development, and neonatal well being. Such studies will result in the accumulation of data pertinent to the reproductive physiology and husbandry of this unique goat strain. As part of the standardizing process, in-depth studies of the nutritional, metabolic, and genetic characteristics of the Pygmy goat will be carried out in cooperation with members of the Animal Science Department of Oregon State University at Corvallis. Studies in transplantation of fertilized ova are planned with a view to developing a means by which the size of the Pygmy goat herd can be increased more rapidly. The African Pygmy goat is a dwarf goat which reaches maturity at six months and has a long breeding season. Its adult weight of 50 pounds and height of 60 cm. make it easy to handle (even by female laboratory technicians), cheap to feed, and economical to house. It is an herbivore, trains easily, and lives naturally in herds subject to accepted procedures of animal husbandry.

Significance to Biomedical Research and Program of the Institute: It is anticipated that the development of this animal for research purposes may result in a preferred alternative to the sheep for fundamental research relative to problems of transplacental diffusion, blood flow during pregnancy, prenatal life, parturition, and neonatal adaptation. The increasing interest in the physiology of the fetal-maternal complex demands the development of an experimental animal with uniform physical and genetic characteristics. It would appear that the Pygmy goat may well fulfill these requirements.

Proposed Course: The contract for the development of a Pygmy goat colony was initiated March 14, 1967, and its period of performance is anticipated to be three years.

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Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Diagnosis and Treatment of Disorders Affecting the Intrauterine Patient PH-43-67-1327
Contractor : Columbia University
Money Allocated: $10,485

Objectives: This contract with Karlis Adamsons, M.D., Ph.D., of the Department of Obstetrics at Columbia University, will result in a document concerned with the diagnosis and treatment of prenatal disorders. It will contain information about:

1. The present state of knowledge related to morphologic and cytogenetic methods in the diagnosis of fetal conditions, metabolic performance of placenta and fetus, amniotic fluid and fetal blood as indicators of fetal circumstance, physical methods in the assessment of fetus, and prenatal treatment including the prevention of Rh disease, intrauterine transfusions, fetal surgery, and therapeutic possibilities of prenatal enzyme induction.

2. What can be expected to be accomplished for fetal welfare by the application of already extensive knowledge and methods.

3. Identification of gap areas where new diagnostic methods should be developed so as to enable the obstetrician to detect deviations from normal intrauterine fetal development.

4. The theoretical identification of possible therapeutic procedures during prenatal life.

5. Types of instruments or equipment needed which are not presently available for the diagnosis and treatment of prenatal disorders.

6. Pertinent bibliography.

Significance to Biomedical Research and Program of the Institute: If major accomplishments are to be made in reducing perinatal and infant morbidity and mortality, increased understanding of problems and causative factors occurring in intrauterine life is needed as well as identification of methods of prevention and/or correction of aberrent conditions. It is anticipated the document resulting from this contract may serve as a stimulus for greater interest and research and provide a base for action.

Proposed Course: This contract concerned with the diagnosis and treatment of disorders affecting the intrauterine patient was initiated June 29, 1967, and its period of performance is anticipated to be 15 months.

Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Study of Selected Aspects of the Psychodynamics of Pregnancy
Contractor : Group Health Association, Inc. PH-43-68-642
2121 Pennsylvania Avenue, N.W.
Washington, D.C. 20037
Money Allocated: $21,296

Objectives: This contract with the Group Health Association will extend the analysis of data already collected as part of the Association's currently active Demonstration Project on Prenatal and Early Postnatal Adaptation so as to result in a scientific report on specific aspects of the psychodynamics of pregnancy, including psychological adaptation to pregnancy, relationship to pregnancy outcome, and subsequent maternal-infant adaptation. The following major areas will be explored:

1. Study of variables in the life history and in the individual personality that are related to the adaptation to pregnancy, labor, and delivery.

2. Investigation of the relationship between the woman's psychological adjustment and pregnancy outcomes, including complications of delivery and initial adaptation to the maternal role.

3. Analyses of the predictive significance of life history and prenatal personality characteristics for adaptation to the maternal role.

4. Analyses of the relationships between maternal characteristics and selected aspects of the postnatal adaptation of the young infant.

Significance to Biomedical Research and Program of the Institute: In the past, major emphasis in the scientific community in the study of perinatal and infant mortality has been upon the study of physiological aspects of pregnancy and their relationship to pregnancy outcome and perinatal and infant mortality. Less emphasis has been directed to understanding psychological factors in pregnancy maintenance, pregnancy outcome, and maternal-infant adaptation or to identifying physiological-psychological relations and interrelationships. A major gap in the area of perinatal and infant survival and well being concerns psycho-social factors of pregnancy and their relationships to pregnancy outcome and perinatal and infant mortality. The analysis of these requested data will result in a document reporting on selected aspects of the psychodynamics of pregnancy. It will also serve the Institute in its development of the behavioral component of the Perinatal Biology and Infant Mortality program.

Proposed Course: The contract for the preparation of a document concerned with a study of selected aspects of the relationship of the psycho-social factors in pregnancy and pregnancy outcome was initiated January 4, 1968; its period of performance is anticipated as twelve months.

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Growth and Development Branch
Contract and Collaborative Research

During the past twelve-month period, special attention has been given to utilizing research contracts for meeting specific, targeted goals of the Program. Thus the total number of contracts administered by Branch staff has increased from 9 in 1967 to 15 at the present time. Several additional ones are under negotiation and should be activated by the end of the current fiscal year. This represents a two-fold increment in funds committed to contract research. A variety of projects, ranging from the development of nutrition terminology for literature storage and retrieval purposes to improved methodologies for studying how children and youths learn to live in the world about them, have been implemented. At the same time, improved monitoring of existing projects has been a major goal of staff. NICHD's largest single contract, devoted to the study of malnutrition and mental development, has just been reviewed in depth by international experts and endorsed as a major interdisciplinary study in an extremely critical area.

Conferences sponsored by the Growth and Development Branch have been as varied as the other phases of our total effort. A workshop on the "Social Aspects of the Socialization Process" was successfully held in December 1967. This was followed by an important exploration of "The Reading Process" which paid particular attention to the causes of and approaches to reading disability. A third conference, this one on "Nutrition and Cell Development," is scheduled to be held in late May. Publications are planned from each of these meetings.

One conference was supported by contract during the last year. This week-long symposium on "Issues in Human Development" brought together biological and behavioral scientists with specialists in urban planning and law to discuss many of the leading issues facing the United States today. A proceedings drawn from the papers and the resultant discussion is currently being edited for publication next year.

Late in FY 1967 the Branch sponsored a conference on the "Use of Subhuman Primates for Human Development Research". From these discussions the Institute learned of the critical need for improved and more reliable sources of primates for developmental studies. Subsequent to this conference a survey was completed of the present and projected needs for primates by investigators presently supported by this Institute. The National Primate Centers and representative other primatologists were included in the survey. The need for timed pregnancies, for animals for contraception studies, for infants with known birth dates and parentage, and for mother-infant pairs was dramatically reinforced. A summary of this survey was presented to NICHD Advisory Council at its March meeting. In conjunction with DRFR, ways for expanding primate production with selected species are currently being sought.

Epitomizing the interdisciplinary approach to nutrition at NICHD is a conference organized by the Growth and Development staff for May, 1968. Its purpose is to explore the development of cells, the factors which modify cell development in different tissues, and the significance of changes in cell

number, size or function in such problem areas as obesity, neurological development, longevity, and the consequences of protein-calorie malnutrition. Nutrition as it relates to liver growth, placental development, hormonal mechanisms, etc., will be explored in depth. The proceedings of this conference will be published in mid-FY 1969.

The area of nutrition and intellectual or neurological development continues to be a major focus. Similarly, interrelationships between nutrition and behavior--whether food habits, psychological performance, self-image concepts or energy expenditure--are being actively pursued. A series of projects on hormonal aspects of nutrient utilization form a significant part of the total nutrition effort.

A major programming effort has culminated in the recently approved but not yet funded contract "Mathematical Quantification of Developmental Electrophysiology of Brain Function (EEG)". The objective of this contract is to identify paremeters of mathematical functions which can define and describe functional stages of brain development and which would reflect the effects of genetic and environmental variations upon the developing brain, e.g., identical twins and impoverished environments.

A major conference on "Social Aspects of Socialization" was held during the year. This conference served to focus attention on several important aspects of the socialization process. Six sessions discussed 1) bio-social perspectives on development, 2) socio-linguistics, 3) role development, 4) socio-sexual development, 5) non-parental sources of socialization, and 6) social structural perspectives on development. A final session emphasized the need for multidisciplinary research on socialization, and repeated the oft-heard endorsement of well-planned longitudinal studies as an extremely important source of information on the socialization process.

Contracts continued to play an important part in the social growth and social context extramural activities. One of the most important contracts is the "Study of Nutrition and Intellectual and Social Development" being conducted by the Institute of Nutrition of Central America and Panama under a contract between the NICHD and the Pan-American Health Organization. This is a longitudinal study of the relationship between protein-calorie deprivation, intellectual development, and the social factors which mediate this relationship. This third year of the contract has seen the development of the final methodology for the project, and the successful piloting of a nutrition supplementation program. A summary of progress on this contract is presented later.

Two contracts in the area of socialization have also moved forward. One of these, concerned with the nature of interpersonal influences, has developed some interesting new "laboratory" methods for the study of influence and, in the analysis of data collected in another study, has shown that children and youth who are primarily "peer" oriented tend to have a significant proportion of their friends outside of the classroom and to engage in more antisocial behavior than do youngsters who are adult oriented. The other project, concerned with the nature and effect of a multitude of socializing agents is currently reviewing relevant theory and research findings, and developing new methodology for the study of socialization. A simulation game, in which

the payoffs for various decisions can be varied for each participant, shows promise of helping to clarify the ways in which youngsters resolve conflicting pressures from various internal and external sources.

In addition to active programming of specific research and training programs in the area of reading, the staff held a conference on "The Reading Process" in New Orleans on February 11-13, 1968. Fifteen researchers representing a wide variety of disciplines concerned with the reading process attended the meeting. Each of the principal participants was encouraged to bring his outstanding graduate student to contribute to the discussion of current research in this area. A book entitled The Speech Process is now being developed.

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Growth and Development Branch
Contract and Collaborative Research

Contract Title: Survey Committee on the Behavioral and Social Sciences (PH43-64-44)
Contractor: National Academy of Sciences-National Research Council
Washington, D.C.
Stephen Viederman
Money Allocated: $25,000 (FY 1968)

The National Institute of Child Health and Human Development is participating with the National Institute of Mental Health, the National Institute of General Medical Sciences and the National Science Foundation in the support of a major study of needs and trends in behavioral and social sciences being conducted by the National Academy of Sciences-National Research Council. The NAS-NRC has appointed a number of working committees in the behavioral and social sciences, and these working groups have commissioned a series of overview papers. With another year of work to be done, there is every expectation that the report will represent a landmark on the current status and future potential of the behavioral and social sciences.

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Growth and Development Branch
Contract and Collaborative Research

Contract Title: Speech Acquisition in the Human (PH43-65-637)
Contractor: The Johns Hopkins University School of Medicine
Baltimore, Maryland
Richard A. Chase, M.D.
Money Allocated: $152,641 (FY 1967)

The primary aim of this research program is to increase our understanding of how children acquire and use spoken language. Major emphasis has been placed on exploring the effects of the language environment on the development of speech production and speech perception capabilities. Much of this work has been concerned with examining in detail the manner in which children under the age of two years acquire the ability to make the voiced-voiceless distinction. These studies have involved comparisons between the same children at different ages as well as comparisons between children raised in different language communities supplemented when necessary with data from adult speakers. In addition, comparisons have been made between normal-hearing and hearing-impaired children.

The contractor has also investigated the speech perception and production capabilities of older children. A cross-language study employing computer generated speech sounds has explored the perceptual capabilities of children while another study has been concerned with the ability of young children to produce and discriminate foreign speech sounds. A third study has explored the possibility of using the Visible Speech Translator for teaching novel vowel sounds to children between four and five years of age. In addition, the reseachers have continued their work on the computer synthesis of vocalizations produced by infants and young children.

During the past year some new work was initiated. One of these projects was designed to explore the development of word formation in children. A second project examined identification and discrimination functions for synthetic speech sounds presented in each ear separately, and a third explored the effects of modulated pure tone on delayed auditory feedback.

As in previous years the contractor has made use of several facilities outside of his laboratory. Within The Johns Hopkins Medical Institutions he has obtained subjects from the Hospital Staff Nursery School and from the Hearing and Speech Center. He has also made use of the Information Center for Hearing, Speech, and Disorders of Human Communication. Outside of The Johns Hopkins University he has maintained a close collaborative arrangement with the Haskins Laboratories in New York City. All of the work in speech synthesis has been done with the synthesizer recently installed at Haskins Laboratories.

The Institute has now under review an application from the contractor for program project support of this research. An orderly transition from contract support to research grant support is anticipated during the fourth year of the program.

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Growth and Development Branch
Contract and Collaborative Research

Contract Title: Study of Relationship Between Nutrition and Behavioral Development (PH43-65-640)
Contractor: Pan American Health Organization, Institute for Nutrition in Central America and Panama
Cipriano A. Canosa, M.D.
Money Allocated: $330,000 (FY 68)

This contract has been designed to contribute additional knowledge of the relationship between nutritional status and physical, psychological, and social growth. The plan calls for the matching of three pairs of small villages (aldeas) of approximately 650 inhabitants each, on a variety of sociocultural variables. In one village of each pair a dietary supplementation program will be introduced, with special efforts made to include in this supplementation program every preschool child and pregnant woman in the village. Incaparina, a high protein food supplement, will be made available to every person in the village who wants it. In the control villages, medical service will be offered, but no food supplementation will be provided. Thus a sample of well-nourished preschool age children can be compared on relevant dimensions with a sample of medically treated preschool children raised under the dietary conditions characteristic of the area. Children will be followed from birth until about six years of age, at which time effects of differences in nutrition should be clear.

The psychological testing program has involved the development and standardization of tests of intellectual development and cognitive development, since no appropriate Spanish language tests suitable for use with this population existed. The tests include a Gesell-Bayley like infant scale, a test of vocabulary, perceptual analysis, memory, conceptual reflection versus impulsivity, selectivity of attention, conceptual richness and conceptual strategies. The bio-medical tests include body size and weight, maturational level as determined by bone development, and the incidence and duration of disease. Neurological development will be routinely evaluated. The social data includes socio-economic characteristics of the family and the village, social competence, ecological distributions of the population, etc. The nutritional studies include a careful evaluation of the diet of the participating families, along with careful measurement of the amount of Incaparina received by each subject child. Medical care will be provided to the families in all participating villages, regardless of whether or not the family is participating in the study itself.

A thorough review of the bio-medical aspects of the program in the Spring of 1968 by a group of authorities in human growth and development led to an enthusiastic endorsement of the study plan. This group of consultants from leading universities and research organizations throughout the world spent a full week reviewing in detail the plans and pilot findings of the project.

This was followed by a week of final evaluation of the psychological and social methodology by the permanent behavioral science consultants.

A full year of experience with a pilot nutrition supplementation program in one village indicates that the most relevant persons (pregnant women and preschool children) will participate in such a program regularly through time. Although there are some seasonal differences in participation--with a drop during the coffee picking season when whole families are away from the community all day long--an acceptable level of participation has been reached. Plans are being developed which will increase the level of participation, particularly among the very young children.

Initial selection of the villages to be included in the study is well underway. Two villages have already been matched on relevant biomedical, psychological, and social characteristics, and initial data for matching has been gathered on twelve other communities from which the other four villages will be selected. It is anticipated that all three pairs of villages will be established and "on stream" by mid-FY 1970.

Contract Title: The Psychologist in the School: Exploration of a new role for the study of child development in relation to the educative process. (PH43-65-996)
Contractor: Bank Street College, New York City
Herbert Zimiles, Ph.D.
Money Allocated: $13,572 (FY 1967)

A small contract with the Bank Street College of Education on the role of the school psychologist is in its final year of support. Several explorations of new ways in which a school psychologist can be used as a resource to meet the educational needs of schools, pupils, and teachers, have been made. These new roles include activities such as serving as a consultant on curriculum developments related to the cognitive developmental stage of the student, serving as a general resource for the teaching staff during faculty meetings, and looking for additional, non-individually therapeutic ways in which the school psychologist's training and expertise can be of use to the total school. A final report of this contract is expected in the near future.

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Growth and Development Branch
Contract and Collaborative Research

Contract Title: Longitudinal Study of Cognitive Growth in Infants (PH43-65-1009)
Contractor: Harvard University, Cambridge, Massachusetts
Jerome Kagan, Ph.D.
Money Allocated: $51,528 (FY 1967)

This contract concerned with the early antecedents of cognitive development has focused on social class differences in early maternal behavior and its significance for the development of cognitive functions during the first three years. Children of middle and lower class status have been studied at 4, 8, 13 and 27 months. The first phase of data collection has been completed. Among the major findings are:

1. During the first year of life, there are significant class differences in the development of attentional processes in infants. These differences are evident by four months of age. The relationship of class to attentional functions is greater for girls than for boys.

2. Class differences are also evident by four months of age in infant's responses to aberrant facial schema as measured by cardiac deceleration. Upper middle class infants are more clearly sensitive by four months of age to variant facial schema. These differences between the upper middle and the lower social class groups are thought to be related to differences in the two groups in amount of face to face contact with the mother.

3. Class differences were also found in the incidence of separation anxiety in eight-month-old infants. As measured in terms of latency of crying when the mother left the room, separation anxiety is less frequent among lower class infants at eight months of age than among middle class infants. This difference appears to be related to differences in maternal care in the two groups.

This contract has achieved the original goals and is being terminated in FY 1968. A final report is in preparation. An application for a program project has been submitted to NIH to extend these findings into additional groups and situations.

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Growth and Development Branch
Contract and Collaborative Research

Contract Title: Developmental Trends in Cognitive Behavior (PH43-65-1011)
Contractor: Fels Research Institute, Yellow Springs, Ohio
Eugene J. Gollin, Ph.D.
Money Allocated: $10,628 (FY 1968)

The objective of this project has been to provide a systematic picture of the trends in the development of cognitive behavior in preschool and school age children. The study has focused on the exploration of the developmental shift in cognitive behavior from dependence upon sensory-perceptual and motor processes to reliance on inferential or conceptual representation and manipulation. Specifically, studies on oddity-problem learning, probability learning, perceptual distinctiveness, and perceptual learning and recall have been conducted. Development of new research methodologies has been a primary goal of the contract.

Some of the findings from this research indicate that preschoolers tend to perseverate responses to a "position" clue to a greater extent than kindergarteners and that this response tendency appears to be entirely absent in the second grade groups. Furthermore, kindergarten children are able to overcome early position preference more effectively than preschool children.

The ability to verbalize solutions was found to be significantly different between the preschool, kindergarten and second grade groups (5, 20, 70 percent of samples, respectively). An analysis of the content of the verbalizations also indicates significant differences between the groups which suggests different cognitive strategies between the groups even though terminal performance was not significantly different between kindergarten and second grade groups.

Additional results confirm the hypothesis that the cognitive behavior of young children is dependent upon the sensory qualities of the stimulus and associated reinforcements, whereas in older children shifts in cognitive style to a conceptual-verbal as opposed to perceptual-motor approach result in solutions that are not as dependent upon the perceptual distinctiveness of the stimuli nor the associated reinforcements as it is for young children.

The principal investigator is moving to the University of Colorado where this research will be continued for a terminal year. The objectives of the one year contract at Colorado will be to assess the sensitivity of response measures, developed under this contract at Fels Research Institute, to the impact of impoverished environments upon cognitive development and to initiate exploratory studies which would relate these cognitive measures to parameters of brain electrophysiology, as obtained by Dr. Metcalfe and Dr. McCandless.

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Growth and Development Branch
Contract and Collaborative Research

Contract Title: Evaluation of Harvard-Florence Longitudinal Study (PH43-66-938)
Contractor: Michigan State University, East Lansing, Michigan
Lucy Rau Ferguson, Ph.D.
Money Allocated: $19,000 (FY 1965)

The major objectives of this contract were to assess the potentialities for continuing the Harvard-Florence Project, for further analyses of the data and to consider future research possibilities with this population. This cross-cultural longitudinal study of physical and psychological development of 330 males of Italian background in the United States and in Italy was initiated in 1956.

To assess critically the future research possibilities with this sample, the following procedures were carried out:

1. Specification of the characteristics of the study sample in Boston, Rome and Palermo.

2. Tabulation of the psychological and social measures obtained on this sample at various points in time throughout the course of the study.

3. Preliminary data analyses to evaluate the probable yield of the project files on questions relevant to the behavioral sciences.

On the basis of the evaluations of the data and preliminary analyses, both the limitations and potentialities of the existing data were clearly identified. Some potentially fruitful areas for analyses of the current data were suggested. For example, there are indications of interesting differences between the three cultures in vocational aspirations, political interests, attitudes towards school, attitudes towards the family, and affiliative behavior. Other analyses suggest that it may be useful to explore cross-cultural differences in the structure of motives and the meaning of fantasy in the three groups. A major publication has been outlined which would incorporate these analyses along with contributions from a number of visiting scientists who have spent some time with the project.

Specific recommendations regarding future research were made, and recommendations for the staffing of the project were specifically delineated. Among the research problems which might be investigated with this sample were the following: the relationship of differences in closeness of family ties in the American and Italian samples to patterns of affiliation, extrafamilial friendships, and autonomy; cross-cultural differences in attitudes towards aggression and in sources of control of aggressive impulses; and differences in social and moral values.

Growth and Development Branch
Contract and Collaborative Research

Contract Title: To Conduct a Conference on the Longitudinal Method in Study of Growth and Development (PH43-66-949)
Contractor: Temple University, Philadelphia, Pennsylvania
Victor C. Vaughn, M.D.
Money Allocated: $14,950 (FY 1965)

This conference, long in the planning phase because of its size and complexity, was held as scheduled in Philadelphia November 27-30, 1967. Investigators from the biomedical and behavioral disciplines including such outstanding leaders as Rene Dubos, Stanley Garn, Jerome Kagan and many others presented stimulating accounts of current research and significant unresolved health, social and developmental issues. Emphasis throughout the conference was on questions requiring longitudinal study with particular reference to children living in urban environments.

The conference format of half-day sessions initiated by a provocative paper and followed by specific respondents, panel discussions and open discussion was generally successful and in many instances resulted in an enthusiastic exchange of ideas. There were seven sessions dealing with the following topics: Earliest Influences; Physical and Chemical Growth and Development; Socialization; Cognition and Learning; Family, Adolescent and Culture; The City; and Institutions, Economics and Law.

A highlight of the conference was the presentation and discussion of a movie entitled, "The Jungle," which was produced entirely by the 12th and Oxford Streets Film-Makers. "The Jungle" is a story of survival in the slums of north Philadelphia and depicts in vivid detail the physical and social environment, and the structure and habits of a typical street gang.

A stenotype transcript of the conference is available and each of the participants has had the opportunity to review his contributions. The proceedings are being edited with the hope that they will be made widely available in either a summarized paper or a more detailed monograph.

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Growth and Development Branch
Contract and Collaborative Research

Contract Title: Review of Child Development Research (PH43-66-959)
Contractor: Society for Research in Child Development
Madison, Wisconsin
Margaret Harlow, Ph.D.
Money Allocated: $40,350 (FY 1966)

This contract with the Society for Research in Child Development has as its basic purpose the preparation of a volume containing integrative reviews of recent advances in knowledge in child development. These reviews, prepared by experts in child development, will be designed to highlight significant research problems as well as to provide a conceptual framework for relating research findings to problems of practice in pediatrics, clinical psychology, social work and other professions involved in services for children. The volume is to be ready for publication during FY 1969. First drafts of review chapters have been prepared on the following topics:

1. Development of infant-mother attachment.
2. Control of aggression.
3. Parent participation.
4. Social class and race as determinants of differences in child cognitive abilities.
5. Educational policies and philosophies.
6. One-parent families.
7. Effectiveness of environmental enrichment programs.
8. Adoption procedures.
9. Influence of the father.
10. Effectiveness of behavior modification techniques.
11. The child and the law.
12. The role of early experiences in development.
13. The ecology of child behavior.

Growth and Development Branch
Contract and Collaborative Research

Contract Title: Social and Psychological Ecology of Peer Groups (PH43-67-1330)
Contractor: Cornell University, Ithaca, New York
John Condry, Ph.D.
Money Allocated: $13,255 (FY 1967)

This contract had as its original goal the exploration of the social-ecology of peer groups using methods to be developed under the contract. Early emphasis was to be placed on the distribution and nature of naturally occurring groups of children and adolescents. During the first year of operation, however, the focus of attention shifted towards more controlled explorations of the influence process as a necessary first step in understanding both the ways in which peer groups have influence over their members and the reasons for a differential orientation by children towards adults or peers. Several ingenious laboratory experiments have been designed to further clarify the nature of interpersonal influences. Analyses of collected data suggests that youth who are peer-oriented have most of their friends outside of the school-class, and are more likely to participate in socially unacceptable behaviors than are adult-oriented youth.

Contract Title: A Study of Multiple Socialization Inputs (PH43-67-1332)
Contractor: University of Michigan, Ann Arbor, Michigan
Ronald Lippitt, Ph.D.
Money Allocated: $50,000 (FY 1967)

A two-year contract to develop theory and methods relevant to the ways in which children and youth perceive, integrate, and respond to multiple socialization inputs is proceeding on schedule. At the end of the first year a good deal of relevant literature has been reviewed, and a series of new instruments for looking at the socialization process are being developed. Most notable of these is a simulation model which makes public the various factors which a child must take into account in making a decision, and which can be varied to take into account different influence sources (i.e., peers, parents, siblings, etc.) A conference to be held later this spring will bring together a number of experts in the field to review the work accomplished so far and to suggest directions for future work.

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Growth and Development Branch
Contract and Collaborative Research

Contract Title: Guide to Nutrition Terminology (PH43-67-1449)
Contractor: Vanderbilt University, Nashville, Tennessee
Elizabeth Neige Todhunter, Ph.D.
Money Allocated: $21,250 (FY 1967)

Nutrition is a subject which draws upon many other disciplines for methodologies and information. This is clearly evident when it is recalled that nutrition has a major impact on health during the total life cycle of the individual, is a major contributor to the cause and treatment of many disease processes, relates directly to the problem of world population growth, and is intimately intertwined with economics and social development throughout the world. Nevertheless, there is no universal nor satisfactory method for tapping or evaluating the wealth of information available in the publications from the interlocking disciplines which contribute directly to the broad field of nutrition.

This contract is jointly sponsored by the National Institute of Child Health and Human Development and the National Institute of Arthritis and Metabolic Diseases. The goals of the contract are:

1. To develop a guide to nutrition terminology that will provide NIH and the general scientific committee with a standardized terminology that will facilitate indexing, storage, and retrieval of nutrition information. This guide will provide a formulation, when incorporated into the MeSH terminology of the MEDLARS system, which will serve as a basis for a recurring bibliography of nutrition literature.

2. To develop a list of journals, and other periodic publications which will serve as a resource for nutrition research information. This list would be accompanied by a statement of the scope of nutrition coverage and frequency with which nutrition appears in each resource.

During the first year the various systems and terminologies for storing and retrieving biomedical literature have been studied in depth. Particular attention has been given to the MeSH terminology of MEDLARS. A terminology has been developed in preliminary form which consists of thirteen major subgroupings of terms, scope notes and selected definitions have been drafted, and a plan for cross referencing the Guide to other major terminologies has been outlined. This approach has been reviewed by a continuing group of specialists who have endorsed the approach taken; selected subcommittees are now assisting in the delineation of particularly difficult areas.

The second year of the contract will be devoted to refining the terminology--completing the categories, the sub-category word lists, appropriate definitions, and cross-references to other terminology systems. By the end of this contract year the Terminology is to be ready for publication and for use by other scientists and/or scientific libraries.

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Growth and Development Branch
Contract and Collaborative Research

Contract Title: Developmental Electrophysiology of Brain Function and Behavior (PH43-67-1451)
Contractor: University of Utah, Salt Lake City, Utah
Edward C. Beck, Ph.D. and Robert E. Dustman, Ph.D.
Money Allocated: $90,085 (FY 1967)

The objectives of this contract are to establish basic normative data on cortical evoked potentials (CEPs) from the visual, auditory, and somatosensory modalities on normal children age 4-16; to conduct studies on the effects of impoverished environments on children's brain function, as measured by CEPs, and to conduct studies on identical twins. Behavioral studies are being initiated to relate to the CEPs.

The first year has been very successful. The PDP-9 computer has been acquired and made operational. Thirty children ages 5-7 have been tested on all these sense modalities to determine whether the significant increase in amplitude of the CEP for this age range exist in other modalities beside the visual. Results from the somatosensory system confirm the earlier findings obtained with the visual sensory system. Thus, the fact that the age span of 5-7 years is a critical period is given further support by data from this contract.

In a study of 40 children, 20 with an average I.Q. = 80 and 20 with an average I.Q. = 140, it was found that the high I.Q. children had significant inter-hemispheric differences in the amplitude of the CEPs whereas the low I.Q. children did not show such differences. Additionally, the responses of the bright children were found to be significantly more stable than those of the dull children. If these phenomena can be validated in additional samples, then a major contribution would have been achieved toward understanding brain function and organization as it relates to intellectual capacity and function.

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Growth and Development Branch
Contract and Collaborative Research

Contract Title: Organize, Analyze, and Document Data of Healthy Subjects (PH43-68-634)
Contractor: University of Colorado, Denver, Colorado
Robert W. McCammon, M.D.
Money Allocated: $108,539 (FY 1968)

The objectives of this contract are to organize, analyze and document selected data resulting from 36 years of study of 200 healthy individuals by the Child Research Council (CRC). While the CRC has extensive psychologic, physiologic and other types of data on this group of subjects, the contract is limited to the anthropometric measurements, the radiologic data, the nutrition studies and the health records. No new data are being collected under the terms of the contract.

A major goal of the contract will be to develop a computer-based storage and retrieval system which will facilitate the utilization of reliable, edited data by the CRC and other qualified investigators. By the end of the first contract year, all methods and changes of procedures of data collection will be recorded together with a complete documentation of all phases of computer processing. In addition, summary statistics for the different types of data will be available including frequency distributions, means and standard deviations or percentiles where appropriate and simple correlations. Simultaneously, more elaborate statistical approaches designed to exploit the longitudinal nature of the data will be under consideration for subsequent implementation. Finally, efforts will be made to conceptualize problems uniquely suited to investigation using the data on hand.

The objectives of this contract reflect the recognition that some of the early global long-term longitudinal studies of child development have compiled valuable data which would be extremely expensive and time consuming to duplicate, if indeed they could be duplicated at all. However, these data have not always been analyzed as extensively as desirable. The present project is designed to obtain full value from the CRC data resource. The kind of data files and analyses proposed, if developed with sophistication, should serve as a model for other current and future longitudinal studies of human development. In fact, the results of this effort are likely to serve as an important basis for determining future directions of certain aspects of the Growth and Development Program.

Growth and Development Branch
Contract and Collaborative Research

In addition to the active contracts, several new contracts have been approved on the basis of scientific merit and program relevance. The following three are currently under negotiation for funding late in FY 1968.

Contract Title: Mathematical Quantification of Developmental Electrophysiology of Brain Function (EEG)
Contractor: Tulane University, New Orleans, Louisiana
Bernard Saltzberg, M.Sc.
Money Allocated: Estimate $209,118 (FY 1968)

The primary objective of this contract is to determine whether functional stages of brain development can be identified, described, and quantified by means of mathematical analytic techniques. Time series analysis methods will be applied to electrophysiological signals collected from children. Pattern recognition procedures will then be applied to the descriptors derived from the time series analyses. In addition, efforts will be directed toward determining the sensitivity of mathematical procedures in discriminating among special populations, e.g., the impact of an impoverished environment on brain development, identification of children with learning disorders, and identification of human genetic variations in fraternal and identical twins.

The analog data input for this project is being collected by Doctors Beck and Dustman under an existing NICHD contract with the University of Utah (PH43-65-1011). The data will be forwarded to Professor Saltzberg at Tulane for time series analyses. This collaborative arrangement takes advantage of a pool of data already acquired for other purposes (evoked potential studies) and, therefore, provides substantial savings of both time and money by eliminating the need for a separate data collection effort.

Contract Title: Foster Care: Potential and Self and Public Images
Contractor: Social Psychiatry Research Associates, San Francisco, Calif.
Dorothy Miller, D.S.W.
Money Allocated: Estimate $61,159 (FY 1968)

This eighteen-month contract will support analyses of previously collected data on the self and public images of foster parents, explore the potential which exists in the general population for the recruitment of new foster parents, and explore correlates similar and different child training practices among Negro and White females, and among foster and non-foster parents.

The specific data to be analyzed will include those bearing on the following:

1. The extent to which women in the genaral population show potential for caring for children not their own, including the extent to which those who are high on potential are similar to or different from existing foster parents.

2. The social, demographic and child training characteristics of foster parents as compared to the random sample of Baltimore women.

3. Validation of existing criteria for selection of foster parents, particularly in terms of their values, child training practices, and family organization.

4. White-Negro differences in child training practices, controlled by social class. Although a good deal has been written about child training practices among white families, little published information exists on child training among Negroes.

Contract Title: Cross National Research on the Family
Contractor: Western Reserve University, Cleveland, Ohio
Marvin Sussman, Ph.D.
Money Allocated: Estimate $70,805 (FY 1968)

The specific objectives to be studied include:

1. To develop methodology and theory for a cross-national study of the relationships between societal scale, family structure, and socialization, and to put the developed theories and methods to the empirical test. Of considerable concern will be the development of theoretically equivalent measures of important variables that may be expressed in different ways in different cultures.

2. To develop and publish a series of monographs and papers dealing with theories of the middle range in societal scale, family organization, socialization, etc. and their interrelationships.

3. To conduct pilot studies to test the theories. These pilot studies will also serve as the tests of methodological developments and innovations which will be necessary to truly utilize cross-national data.

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Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Title: Regional Cooperation in Mental Retardation Research

Contractor: Western Interstate Commission for Higher Education (WICHE)

Money Allocated: $49,757

Objectives: The basic contract with WICHE, now entering its third and final year, is for the conduct of epidemiological research in mental retardation on the resident populations of 13 western institutions. Its specific aims are:

1) Continuation of Regional Joint Data Collection Project to provide longitudinal data on the institutional populations and to further refine and enhance the utility of the data.

2) To stimulate cooperative and independent research efforts between and among academic and institutional communities and to capitalize on research potentialities of the data.

3) To facilitate communication between institutions and to disseminate and publish information obtained on a broad scale.

During the final year of the contract the objectives will be:

1) Develop a rigorous, objective description of the regional population of institutionalized retardates in the western states.

2) Develop a set of detailed research hypotheses and problems inherent in this population.

3) Develop descriptions of present and potential implications for increased service to the population.

Methodology: A variety of methods are being utilized in carrying out this project, including:

1) An interdisciplinary Research Advisory Group to improve data collection forms and advise on other aspects of the project.

2) An assessment of each institution's interest and capacity for research and the provision of staff consultation to promote sound research.

3) The development and publication of a current inventory of research projects in the western states.

4) The sponsorship of small study conferences for groups of researchers to facilitate cooperative research, planning, and implementation.

Results: The Joint Data Collection Project now includes 20 state institutions and more than 26,000 patients. The population profiles now available offer rich resources for epidemiological and other type studies.

The data collected is already being used by institutions for program refinement and small scale investigations.

Three publications have been prepared since the last report. The first, A Regional Directory of Research in Mental Retardation (Preliminary), contains informative reports of research in progress or recently completed in the West. The second publication was a brochure describing the Joint Data Collection Project and the data available. The third publication, 1,500,000 Bits of Information...Some Implications for Action, contains the proceedings of a symposium conducted at the ninety-first annual meeting of the American Association on Mental Deficiency on May 18, 1967, Denver, Colorado.

The participation of institutions has made them increasingly aware of the potential uses of data processing equipment and of the concept that research and service are compatible goals. Efforts are also being made to foster associations between institutions and the universities. Distribution of information about the program through formal publications and professional meetings is expected to bring about a national concern in institutions for research in mental retardation.

A standard set of statistical programs are being developed for routine analysis of data. These may include longitudinal studies of population characteristics, cluster or factor analyses, distribution of specific diagnostic entities, etc. Satisfactory progress is being made on the collection and analyses of population statistics and on the promotion of research capabilities within institutions for the retarded.

Contract Title: Prevention of Mental Retardation: The Lesch-Nyhan Syndrome

Contractor: Federation of American Societies for Experimental Biology

Money Allocated: $9,957

Objectives: Under this contract the Federation, in co-sponsorship with the University of Vermont and the Vermont State Department of Health, shall manage and conduct a conference on the subject Prevention of Mental Retardation. The specific disease entity under consideration shall be the Lesch-Nyhan syndrome with the following discussion aims:

1) Is mental retardation a true manifestation of the disease?

2) What is the epidemiology of this disease?

3) What are its social and psychological consequences to the siblings and their parents?

4) Are there experimental models, using animals, which could be utilized in more basic studies? What therapeutic measures could be developed and applied on a wide-scale basis?

Methodology: A three day conference attended by thirty-three experts in the biomedical and behavioral science fields and conducted to assure full discussion and closure of the research issues involved.

Results: The conference was held October 4 through October 7, 1967 at Stowe, Vermont. The discussions were conducted as planned and provided clarification of the research issues presented by the syndrome. Publication of the conference proceedings is anticipated in the May-June issue of "Federation Proceedings."

Contract Title: A Study of Medical Care Practices in Institutions for the Mentally Retarded

Contractor: University of Michigan, School of Public Health

Money Allocated: $90,624 (for 15 month period)

Objectives: This contract is for a developmental study to lay the foundation for a broad scale assessment of medical care practices in institutions for the retarded. It is intended to analyze the relationships between characteristics affecting medical practice such as the legal base for medical care programs, institutional environment, professional staffs, patient populations, and other significant variables. The significance of the project lies in its usefulness in providing an informed basis of the factors influencing medical care for national and local planning and for the development of standards.

Methodology: In preparing a detailed research plan the contractor will: review pertinent research; develop definitive study concepts and design; convert these to appropriate operational form; field test methods; prepare a final proposal for a national study.

Results: This project is several months behind the proposed time schedule, but is making satisfactory progress toward its stated goals. Sampling procedures and the reconceptualization and reformulation of the study's scope and design have now been completed. Data collection instruments and procedures are being field tested for purposes of further refinement and incorporation in a more definitive study for later submission.

Contract Title: Experimental Study of Mental Retardation Classification System

Contractor: American Association on Mental Deficiency (AAMD)

Money Allocated: $43,654

Objective: This contract with the AAMD is for a study to improve the completeness, uniformity, and accuracy of the existing mental retardation classification system.

Methodology: In the conduct of this work, the AAMD will:

1) Select a representative group of state institutions and specialized clinics to participate in an appraisal, revision, and field testing of the classification system. Contacts will be initiated by mail, personal visits, and group conferences.

2) Systematically analyze and attempt to resolve problems encountered in use of classification system.

3) Secure statistical measures of reliability on the use of the classification system by submitting clinical descriptions of selected cases to be diagnosed, classified and coded.

4) Analyze samples of diagnostic, classification, and coding records for completeness of data and coding procedures used.

5) Develop plans for a "Factor Analytic Study of Behavioral Data."

Results: This project, first initiated in February 1967 is now into its second phase of operation. Based on a preliminary analysis of early returns from participating institutions and clinics, the sample has been extended and the research design modified. The initial phase has helped to clarify existing problems in the reliability and validity of the current classification system and to identify difficulties encountered in the use of the system. Modifications of selected aspects of the medical classification (genetic and chromosomal disorders) have already been solicited from leading authorities in the field and will be tested by participating agencies. A factor analysis of the adaptive behavior rating scales is also being undertaken, as planned.

NICHD Annual Report
July 1, 1967 through June 30, 1968
Adult Development and Aging Branch
Contract and Collaborative Research

Contract Title: Theoretical Aspects of Retirement

Contractor: American Institutes for Research

Funds Allocated: $18,684.94

This activity is aimed at the development of several theoretical models descriptive of the retirement process. The models will consider several aspects of retirement including the biological, psychological and social. It is anticipated that they will serve to guide research on retirement by providing theories which research can then test. The retirement process and its related physiological, psychological and social factors is an important area of study and may well be basic in the development of many programs in social gerontology.

NICHD Annual Report
July 1, 1967 through June 30, 1968
Adult Development and Aging Branch
Contract and Collaborative Research

Contract Title: Publication of "Current Publications in Gerontology and Geriatrics"

Contractor: Gerontological Society

Money Allocated: $7,940.00

The objective of this contract is to assemble a bibliography of research in the field of aging consisting of approximately 3,000 citations and to publish this quarterly "Current Publications in Gerontology and Geriatrics" in the Gerontological Society's publication "Journal of Gerontology." This bibliographic information has been published in the Journal of Gerontology for many years and provides a readily accessible source of information on research in aging. Eventually when the Aging Information Center of the NICHD is in full operation, the bibliographic service provided by this contractor will be discontinued. The current contract terminates January 14, 1969.

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